



28° SILAE Congress
16th-20th of September, 2019

La Habana, Cuba

ABSTRACTS BOOK



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Edited by

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SILAE

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WELCOME TO SILAE 2019



As Presidents of SILAE 2019 we have the privilege to welcome all participants to the XXVIII Italo-Latin American Congress of Ethnomedicine, which will take place in Havana from September 16th to 20th 2019.

The overall goal of the meeting is to shed light on the importance of medicinal plants, and to disclose, by innovative models and techniques, new potential applications for the treatment or prevention of diseases. Special emphasis will be given to scientific breakthroughs in the area of traditional medicine unveiling new perspectives for the use of native plants that have been poorly studied so far. The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of

many traditional herbal therapies. Our major objectives are to explore the potential in medicinal plants resources, to understand the challenges and opportunities with the medicinal plants sector, and also to suggest recommendations based upon the present state of knowledge for the establishment and smooth functioning of the medicinal plants sector along with improving the living standards of the underprivileged communities. Thanks to the contribution of many worldwide recognized experts, a multidisciplinary approach will be the file rouge connecting sessions on anthropology, ethnobotany, phytochemistry, food chemistry and nutrition, green and analytical chemistry, pharmacognosy and medicinal chemistry, experimental and clinical studies on natural products within a unique and exciting congress.

Like previous meetings of the Italo-Latin American Society of Ethnomedicine, this conference will represent an excellent opportunity to establish new scientific partnerships, to share results from the most recent studies in the field of traditional medicine and to update the current knowledge on bioactive and/or natural compounds and on their applications.

In line with the Congress, a trade-scientific exhibition will be held at Grand Foyer (main lobby) of the Havana International Conference Center.



Luca Rastrelli and Rodolfo Arencibia

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Palacio de las Convenciones de La Habana, Cuba

Havana International Convention Center

Inaugurated in 1979, the Havana International Convention Center is the foremost institution specializing in events and congresses in Cuba. The facility is located only 20 minutes from downtown Havana, in a residential area in the western part of the city and extends over an area of 60 000 square meters.

The center is surrounded by exuberant vegetation which harmonizes with the tropical greenery of its interior gardens.



The Havana International Convention Center organizes, promotes and hosts congresses, conventions, symposia, conferences, national and international meetings, fairs and exhibitions. It has an extensive network of official agencies throughout Latin America, North America and Europe which, in conjunction with associations or companies interested in these services promote and market the activities organized for each of the international events and fairs programmed during the year.

The professionalism and dedication of its workers have made it a leader in the Cuban congress and exhibition industry.



ORGANIZATION

- Italo-Latin American Society of Ethnomedicine (SILAE FUNDATION)
- Ministry of Public Health of the Republic of Cuba (MINSAP)
- Ministry of Agriculture of the Republic of Cuba (MINAG)
- University of Havana (UH)
- University of Medical Sciences of Havana
- University of Salerno, Italy
- National Technical Group of Medicinal Plants
- Cuban Association of Agriculture and Forestry Technicians (ACTAF)
- Cuban Society of Natural and Traditional Medicine (SCMNT)
- Cuban Organization of Biotechnology and Pharmaceutical Industries (BIOCUBAFARMA)
- National Center for Scientific Research (CNIC)
- National Center for Clinical Trials
- State Center for Medicine Control and Medical Devices
- Dalmer Labs
- Institute of Marine Sciences (ICIMAR)
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- Havana International Conference Center
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Congress program

The XXVIII SILAE Congress will begin on September 17th with the Opening Ceremony and Lectio Magistralis, and will continue on September 17th – 20th with lectures, oral and poster presentations. The meeting will include several sessions:



1. Ethnomedicine and ethnobotany
2. Phytochemistry, pharmacology and pharmacognosy
3. Research, development, marketing and marketing of natural products
4. Clinical and ketogenic nutrition
5. Healthy food and food safety
6. Products of the hive: pharmacological and alimentary use
7. Medical Hydrology (mineral-medicinal waters, peloids, climate) and helium thalassotherapy
8. Agroecology, agrobiological and agrotechnology of medicinal plants and products of the hive
9. Natural products in Veterinary Medicine

PROGRAM:



https://www.silae.it/habana2019/habacont/uploads/2019/11/XXVIII-SILAE-15_9_19.pdf



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International Exhibition for Natural Products Innovation

The natural trade meets at the Natural 2019 Exhibition in La Habana

from 16 – 20 September 2019

NATURAL 2019 EXPO SILAE

Are you interested in the latest developments in the world of natural products, natural food, nutraceuticals, natural fabrics, natural cosmetics and care and would like to establish new contacts? Then you are in the right place at NATURAL 2019 EXPO SILAE! And there are even more reasons to come to Havana from 16 – 20 September 2019

Goal

All Latin America and the Caribbean countries and Italy under one roof – the Natural products sector in the whole Latin America Region will nowhere else meet personally in a comparable way. The exhibition is *the get-together* for customers, suppliers, opinion leaders and the public.

NATURAL 2019 EXPO SILAE brings together all medicinal and food plant stakeholders and associations to establish contacts with Scientific Communities, Universities, Research Centres for the pursuit of medicinal and food plants knowledge. The focus will be on products, branding & marketing. There will also be business, interaction and learning opportunities. Parallel to the exhibition the four days SILAE Congress program with international speakers and panel discussions as well as variety of workshops during the exhibition will inform and exchange knowledge about the natural sector of Latin America.

Latin America Natural Market

Latin America is experiencing strong growth in nutraceuticals, dietary supplement, herbal and cosmetic markets. It has the second-fastest growth in the dietary supplements market worldwide, with an average annual increase of almost 5 percent from 2013 to 2017, according to Euromonitor international. The Latin American market is expected to continue growing at 4.3-percent compound annual growth rate (CAGR), with some markets above 8 percent year over year. The top three reasons for the growth are the aging population, increasing health awareness and higher disposable income.

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The NATURAL 2019 EXPO SILAE, a fair-related exhibition, dedicated to being an information and communication platform for the whole of the Latin America region with the exhibition of products and services derived from the results achieved in technologies, projects and experiences. In this occasion, institutions, laboratories and domestic and international firms will have an excellent opportunity to exhibit their products, equipment, technologies, and books linked to the topic of the event.

Furthermore, the meeting will also offer the chance to spend some spare time in a Cuba a country full of art, culture, music, history and passion
Looking forward to see all of you in Havana next September.

EXHIBITOR PROFILE – Companies or Organizations, who are into:

Agriculture, Antioxidants, Aromatherapy, Ayurveda, Baby Care, Beauty, Beverages, Body Care, Cereals, Colors, Cosmeceutical, Cosmetics, Crop Care, Cultivators, Dairy Products, Diagnostic instrumentations, Diet Products, Dietary Supplements, Encapsulated Herbs, Essence Stick, Fertilizers, Fibres, Fitness, Flavoring, Foods, Foreign Companies, Forest Products, Fortified Foods, Fragrances, Fruits, Gluten-free, Health Spas, Herbals, Homeopathy, Honey & Related, Ingredients, Make-up, Marine Products, Massage, Medicines, Mineral Water, Natural Remedies, Naturopathy, Nutraceuticals, Nutritional Drinks, Nuts, Oils & Oilseeds, Packaging, Perfumes, Pesticides, Plant Extracts, Powders, Probiotics, Processing Equipment, Producers, Raw Materials, Readymade Meals, Refrigerated/Frozen, Rejuvenation Centers, Scientific instrumentations, Seeds, Service, Skin, Soya Products, Spa, Speciality Clinics, Spices, Sports Food, Therapies, Traditional Medicines, Universities, Vegetables.



Abstracts Book of

28° SILAE Congress
16th-20th of September, 2019

La Habana, Cuba



- ⇒ INTRODUCTION
- ⇒ PLENARY
- ⇒ ORAL PRESENTATIONS
- ⇒ POSTERS



EDITORIAL

Luca Rastrelli, Mariateresa Russo

FONDAZIONE SILAE

The SILAE FUNDATION (Italo-Latin American Society of Ethnomedicine, www.silae.it) is an international Foundation dedicated to advancing science around the world by serving as an educator, leader, spokesperson and professional association. The fundamental objective of SILAE is to promote research and development into the use of medicinal and food plants in different countries of the World. SILAE welcomes and actively seeks opportunities to work cooperatively, activating and intensifying scientific relations between countries and between SILAE members. Since SILAE was founded (1990) its objective has been set to contribute to the close examination of the themes of great interest and actuality in the context of the relationships between Latin America and the European Union. In addition to this, SILAE aimed to individualize new ways of collaboration between its member countries and other European as well as Asiatic countries to sign accords with intergovernmental organizations. SILAE proposes to establish contacts with Scientific Communities, Universities, and Research Centres for the pursuit of medicinal and food plants knowledge. There are several disciplines that deal with studying medicinal plants each with its own methods and interest but at the same time with a partial view of the phenomenon.

The model of interdisciplinarity is, in our opinion, the most suitable to have a more comprehensive approach to the study of the flora of a given country.

In addition to organizing membership activities, SILAE publishes the SILAE Special Issues, as well as many scientific newsletters, books and reports, and spearheads programs that raise the bar of understanding for science worldwide.

The congresses of SILAE are international events whose organizations are submitted to an International Organizer Committee composed of professors from Italian and Latin America Universities. The Congress cover the topics of: Anthropology and Ethnobotany, Phytochemistry

and Pharmacognosy, Natural Products and Pharmacology, Analytical Chemistry, Chemistry of Food and Nutrition.

The Italo-Latin American Congress of Ethnomedicine arose from the necessity to evaluate the important potentialities of little known medicinal and alimentary plants, typical and traditional plants of the Latin American continent and to provide connections between Italian and other European and Latin-American researchers, with common objectives of research in the areas in which the projects will be articulated. Traditional medicine is used by 85% of the World's population and is of great importance in developing countries. In accordance with the requirements of the World Health Organization, a scientific basis and proof for the use of medicinal plants is required and so the organization of such a Congress provides an important exchange of such information and coordination of scientific activity. Medicinal plants represent an inexhaustible source of natural active products useful for human life, both for therapeutic and curative aspects, that for their applicability in different areas.

This Abstract Book of the 28° SILAE Congress held at La Habana (Cuba) from 16 to 20th September 2019, provided an opportunity for publication of new research lines on medicinal and food plants used in Latin America; this will serve to stimulate the studies in these areas that are extremely important for academia and industry. The Editors would like to thank the contributors who gave so generously of their time and experience and who made this publication a valuable tool for scientists in the field of natural products chemistry and biology.

Food volatilome fingerprinting by biomimetic system and headspace solid-phase microextraction and gas chromatography-quadrupole time-of-flight/mass spectrometry (HS-SPMEGC-QTOF/MS)

Russo, Mariateresa

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Food aroma profile is characterized by a complex mixture of many components at very low concentration which are responsible of food distinctive identity and has a great importance in consumers' preferences. .

Well known is, in fact, that a priming role in eating behavior plays odors and food choice and intake strongly depends on its intensity. Food choices are mainly conditioned by sensory aspects and above all by the aroma of foods, while, only later, other factors, including nutrient content or health aspects, are involved.

The most important aromatic compounds include compounds derived from the pools of carbohydrates, amino acids, fatty acids and include a very large and heterogeneous group of molecules that have both saturated and unsaturated structures, straight chain, branched chain or cyclic characterized by functional groups and/or by the presence of nitrogen and sulfur which can be grouped in relatively few chemical classes, mainly: aliphatic esters, alcohols, aldehydes, ketones, lactones, terpenoids.

As widely known food volatile substances (VOCs) following interaction with human receptors, encode their aroma and taste.

So, the aim of our work was the development of study, with the aim of characterizing the volatiloma fingerprinting of foods by development of a non-targeted volatile metabolomic approach based on GC-QTOF/MS coupled with solid phase microextraction and organoleptic fingerprinting by Ultra-Fast GC, and *biomimetic system*.

Focusing on the aroma profile and volatile constituents, even if gas chromatography/mass spectrometry (GC/MS) is the technique widely used for both qualitative and quantitative analysis, among the combined techniques, attention has recently been focused on the application of high-resolution quadrupole time-of-flight mass spectrometry (QTOF/MS) which allows a more accurate identification. QTOF/MS provides both a high spectral sensitivity with a high mass resolution, and structural elucidation with high resolution spectrum of product ions. GC-QTOF/MS has an application potential in qualitative analysis of great interest. Thanks to these features, which improve selectivity, sensitivity and accuracy, the application of the GC-QTOF/MS in qualitative analysis, seems to be of great interest.

Odour/taste Map fingerprinting has been determined by Ultra-Fast Gas Chromatography (UFGC), an analytical technique used for rapid acquisition and analysis of the sample's headspace which has been shown to be applicable to trace and ultra-trace analysis of volatile and semi-volatile compounds and e-tongue.



Method

Then volatile compounds extraction was carried out by a 50/30 μm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber for autosampler. A GC (Agilent 7890A) coupled into a QTOF accurate mass selective detector (Agilent, 7200) was used to analyze the sample headspace components.

MassHunter software B.08 was used for the control of equipment and data acquisition and The Unknown analysis software was used for tentative compound identification by comparing mass spectra with NIST11 library data.

The e-tongue analysis were performed with the α -Astree electronic tongue (Alpha MOS company), that consisted of seven different liquid cross-selective potentiometric sensors (JB, BA, BB, HA, ZZ, CA and GA) (Alpha M.O.S.), an Ag/AgCl reference electrode (Metrohm, Ltd). The sensors used are chemically sensitive field-effect transistors (chemFET).

Conclusion

Samples of wine obtained from autochthonous *Gaglioppo* vines and craft beers have been used for the development of the integrated volatoloma fingerprints analysis.

The study demonstrated the potential of the integrated approach which is able to provide both quantitative information and relative to the organoleptic perception in relation to the aroma and taste of the products.

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Italy-Cuba cooperation: twenty years of research on medicinal plants

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Higher plants, which have served humankind as sources of biologically active molecules since its earliest beginnings, continue to play a key role in the world health. Compounds from higher plants are of great potential value as medicinal agents, as "leads" or model compounds for synthetic or semisynthetic structure modifications and optimization, as biochemical and/or pharmacological probes.

As a consequence of the renewed interest in the search of new substances from natural sources as potential candidates in the drug development, since 1999 our research group has been involved in investigation of higher plants employed in Italian, Chinese, African and Latino-American traditional medicine.

This report will focus on the results obtained in this last twenty years on chemical and biological study of Cuban flora, as a consequence of an international collaborative study carried out by the University of Salerno and Cuban Research Institute: Instituto de Farmacia y Alimentos (IFAL), Universidad de La Habana, Instituto Nacional de Angiología y Cirugía Vascular, Salvador Allende Hospital, CQF Centro de Química Farmacéutica del La Habana, Instituto de Ecología y Sistemática, CITMA.

New green valorization strategies for extracting terpenoids with anti-neurodegenerative activity from agricultural by-products

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Alzheimer's disease (AD) is a common neurodegenerative disorder associated with age, accounting for approximately two thirds of all cases of dementia and affecting up to 20% of individuals older than 80 years. AD is characterized by a severe memory loss and cognitive impairments due to a multifactorial neurological pathology, one of them being a progressive decline in acetylcholine (ACh) levels in the cholinergic systems¹. Until now, there is no effective treatment for this disease, and a current strategy is to target different factors, such as increase ACh levels through inhibition of the enzymes acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE)² in the brain.

The olive oil industry has a great economic, commercial and industrial relevance in Spain, being olive leaves one of the main by-products and a promising source of bioactive compounds. Several authors^{3,4,5} have reported the biological activity of different terpenoids in these residues; among them, the most abundant triterpenoids in olive leaves (derived from oleanane and ursane) have proved to have an anti-neurodegenerative activity⁵. Therefore, this work was oriented towards the valorization of olive leaves through the systematic and selective supercritical fluid extraction (SFE) of different families of bioactive terpenoids with potential anticholinergic activity.

This work was supported by the project AGL2017-89417-R (MINECO, Spain). Authors would like to acknowledge the Ministry of Economy and Competitiveness for a Juan de La Cierva postdoctoral grant, the Ministry of Education Culture and Sport for a FPU predoctoral grant, and University of Nariño (Colombia) for financial support.

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Estudio de las plantas medicinales del mercado de la galería central del municipio de Tuluá, Valle del Cauca, Colombia

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Introducción

Los estudios indican que en Colombia existen aproximadamente 6.000 especies de plantas medicinales y aromáticas nativas. Gran parte del conocimiento y uso de las plantas se han perdido con los procesos de culturización, con el incremento de la población urbana y sobre todo con el reemplazo de los productos naturales por los de síntesis orgánica (Ramírez et. al., 2008). En la actualidad existen en Colombia 87 comunidades diferentes entre sí en lo relativo a su historia, costumbres, lengua y cultura, las cuales interactúan de manera compleja (relaciones hombre-planta) con cerca de 50.000 especies de plantas superiores, en el contexto natural de un número grande de ecosistemas diferentes (Díaz, 2016). Es necesario el estudio de la identificación sistemática de estas plantas medicinales, del conocimiento de su origen biogeográfico y de las condiciones de comercialización.

Método

Tuluá cuenta con 250.000 habitantes en la parte urbana. En esta investigación se aplicaron encuestas de manera no aleatoria a 45 vendedores de plantas donde se indagó sobre el nombre común, la parte comercializada (hojas, corteza, tallo, flores, frutos, semillas o partes subterráneas), la condición de extracción (silvestre/cultivada), origen biogeográfico, la condición de comercialización (fresca/no fresca/ambas) y usos terapéuticos. Las plantas colectadas se identificaron en el herbario TULV-INCIVA del Jardín Botánico Juan María Céspedes de Tuluá.

Resultados y Discusión

Se identificaron 195 plantas hasta nivel de especie, que corresponden a 170 géneros pertenecientes a 82 familias botánicas. Las tres familias con mayor número de especies citadas fueron: Asteraceae, Lamiaceae y Fabaceae. La venta de hojas correspondió al 37.7 %, la de tallos al 26.3 %, de flores al 17.29 %, raíces al 9.23 %, cortezas al 3.8 %, frutos al 3.13 % y semillas al 2.55 %. El 43% de las plantas que se ofrecen en venta son nativas, mientras que el 57% son introducidas. Los usos etnomédicos de mayor citación fueron los problemas genitourinarios (11.19%), seguidos de problemas del sistema digestivo (9.85%), síntomas o hallazgos anormales (9.40%) y problemas respiratorios (7.61%).



Conclusiones

No existe en Tuluá un ente gubernamental que lleve registros y el control de los volúmenes de material vegetal comercializado.

Se observa una creciente presión de extracción en las poblaciones de plantas no cultivadas. Se necesitan planes de investigación interdisciplinaria e interinstitucional, para la óptima utilización del enorme potencial de los recursos fitogenéticos disponibles.

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Plantas medicinales en el tratamiento del síndrome metabólico (SM), departamento del Valle del Cauca, Colombia

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Introducción

El síndrome metabólico (SM) es un conjunto de varias enfermedades o factores de riesgo cuyo eje causal es la resistencia a la insulina (Barrera et al. 2008). Para la Medicina Tradicional China, el SM abarca las siguientes enfermedades: enfermedad cardíaca, dislipidemia, hipertensión, diabetes tipo II, demencia, cáncer, síndrome de ovario poliquístico e hígado graso no alcohólico (Guetio, 2014).

Método

Para la identificación de las especies vegetales para el tratamiento de enfermedades propias del SM, se utilizó la base de datos PubMed, e información obtenida de las siguientes comunidades locales: etnia indígena guambiana; negra, en el Parque Natural Uramba (Buenaventura); y mestiza, en la Galería Central del municipio de Tuluá, Valle del Cauca, Colombia (Villegas, 2011).

Resultados y Discusión

Las principales plantas para el tratamiento de cada afección son: Enfermedad cardíaca: *Crataegus monogyna* Jacq., *Digitalis purpurea* L., *Ginkgo biloba* L., *Melissa officinalis* L., *Persea americana* Mill., *Curcuma longa* L. Dislipidemia: *Cynara scolymus* L., *Plantago psyllium* L., *Linum usitatissimum* L., *Persea americana* Mill., *Allium sativum* L., *Solanum melongena* L., *Pinus pinaster* Aiton, *Cinnamomum zeylanicum* Blume y *Cucurbita máxima* Duchesne. Hipertensión: *Allium sativum* L., *Achillea millefolium* L., *Salvia palifolia* Kunth y *Equisetum giganteum* L. Diabetes tipo II: *Curatella americana* L., *Physalis peruviana* L., *Smilax sonchifolius* (Poepp.) H. Rob., *Momordica charantia* L., *Bauhinia variegata* L., *Bidens pilosa* L., *Petroselinum crispum* (Mill.) Fuss y *Sechium edule* (Jacq.) Sw. Demencia: *Ginkgo biloba* L., *Uncaria tomentosa* (Willd.) DC., *Panax ginseng* C.A. Mey., *Vitis vinifera* L. y *Citrus reticulata* Blanco. Cáncer: *Petiveria alliacea* L., *Apium graveolens* L., *Croton lechleri* Müll. Arg., *Uncaria tomentosa* (Willd.) DC., *Annona muricata* L. y *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier. Síndrome de ovario poliquístico: *Cimicifuga racemosa* (L.) Nutt., *Ruta graveolens* L., *Vitex agnus-castus* L., *Aloe vera* (L.) Burm. f., *Occimum basilicum* L., *Achillea millefolium* L. y *Matricaria chamomilla* L. Hígado graso no alcohólico: *Sylibum marianum* Gaertn., *Baccharis latifolia* (Ruiz & Pav.) Pers., *Raphanus sativus* L., *Phyllanthus niruri* L., *Verbena officinalis* L., *Bidens pilosa* L., *Peumus boldus* Molina y *Curcuma longa* L.



Conclusiones

Las afecciones que hacen parte del SM pueden ser tratadas con plantas medicinales de uso local, avaladas por investigaciones de alta calidad científica; el tratamiento ortodoxo de patologías del SM no excluye el uso de fitofármacos. La integración de diversas prácticas médicas es conveniente en el manejo del SM.

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Phytochemical study and evaluation of the antioxidant capacity of the flowers *Fuchsia boliviana* from Colombia

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Introduction

Fuchsia boliviana (Onagraceae) known as Zarcillejo or Platanillo, is a species used in an ornamental way, presenting little or no application from ethnomedicine (Davidse, Sánchez, Knapp & Cabrera, 2009), however *Fuchsia* genus has been reported with diverse biological activities such as antimicrobial, anti-inflammatory and antitumor cases such as *F. fulgens*, *F. splendens*, or *F. procumbens*, and with reports regarding its chemical composition of some secondary metabolites of the anthocyanidin type such as peonidin, malvidin, and delphinidin and some flavonoids as quercetin, apigenin, and lutiolin (Ducrey, Wolfender, Marston & Hostettann, 1994, Parra, 2013); *F. boliviana* is native to southern Bolivia, found in a mountain fraction and Andean forest along the eastern flank of the central Andes, in Colombia it is distributed in the Center and Northeast of the country, in the departments of Choco, Antioquia, Caldas, Santander, Tolima, Boyacá and Cundinamarca. The present work was oriented to the study of the chemical composition of flowers of *F. boliviana* and the evaluation of its antioxidant activity by the methods of DPPH and ABTS.

Method

The collection of the flowers of *Fuchsia boliviana* was made in the city of Bogotá-Colombia (N 4 ° 36'40.41"W 74 ° 3'55.19"), 2000 g of dried flowers were subjected to cold maceration with ethanol 96% obtaining the total extract called E.EtOH Fb.F (19.57 g); from this a liquid-liquid partition was made with solvents of different polarities obtaining fractions of heptane (Hep), dichloromethane (DCM), ethyl acetate (AcOEt) and a hydroalcoholic residue. For the isolation and purification of the fractions, chromatographic methods were used, such as column chromatography (CC), preparative thin layer chromatography (CCDP) and for monitoring the subfractions obtained, thin layer chromatography (CCD), which allowed the isolation

of five compounds and a mixture which were tentatively identified by gas chromatography coupled to mass spectrometry (GC-MS). The determination of the antioxidant capability was determined by using the DPPH and ABTS methods where different concentrations of ethanol extract were tested; also the fractions of Hep, DCM and AcOEt were tested, as well as the statistical analysis of the antioxidant capability (IC₅₀ and inhibition percentage) was accomplished according the number of agreed reports by three times (n=3) ensuring correlation coefficients and variance analysis for each test.

Results/Discussion/Conclusion

The comparison of the spectrums of the obtained masses allowed to identify five isolated compounds of the flower *F. boliviana* named as ethyl palmitic ester acid, eugenol methyl ester, oleanolic acid, galic acid, and 1,2,3- bencenotriol; in addition to a mixture of compounds made of 1-(2-butoxiethoxy) ethanol and palmitic acid; the evaluation of the antioxidant capability allowed to identify that the ethanolic extract, and the fraction of DCM recorded percentages of inhibition higher than 40% taking as a reference the antioxidant capitation of Trolox in the free radical of DPPH and ABTS.

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Ethnobotanical profile of *Sclerocarya birrea* Hochst. Subsp. *Caffra* (Sond) Kokwaro in Muswodi, Limpopo province, South Africa

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Introduction

Understanding the uses of indigenous plants that are of economic importance to local communities is very much important in rural development strategies. *Sclerocarya birrea* commonly known as 'marula', is an indigenous fruit tree species that is widely used in South Africa. It belongs to Anacardiaceae family. The purpose of the study was to unravel the impact of the utilization of *Sclerocarya birrea* species on its population.

Method

Ethnobotanical utilization of the species was collected from thirty informants (23 females and 7 males) using semi-structured interviews. Convenient sampling technique was used to select research participants.

Results/Discussion/Conclusion

The fruits are the most utilized part (63%) followed by the barks (24%) and the leaves (10%). Most of the informants indicated that they utilize the fruits in juice (30%) and wine (30%) making. The species was also reported to be used in traditional medicine (6%). This study clearly brings to light the deepened utilization of *Sclerocarya birrea*. In conclusion, it suggests that a management plan be put in place in communal areas to monitor the harvesting of *Sclerocarya birrea* since it is a protected species in South Africa.

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Traditional uses of the remarkable root bark hairs of *Lannea schweinfurthii* var. *stuhlmannii* (Anacardiaceae) by the *Vhavenḁa*, South Africa

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Introduction

Lannea schweinfurthii var. *stuhlmannii*, an African tree distributed from Sudan southwards to South Africa, has its thicker roots covered by an unusual furry layer of hair-like structures originating from the periderm. The hair-like structures harvested from this layer, known in the vernacular *Tshivenda* as *vhulivhadza*, is a widely used and traded natural product in Limpopo Province, South Africa.

Method

Data was collected through ethnobotanical surveys and literature review. Surveys were conducted among the *Vhavenḁa* (people) found in Vhembe District, Limpopo Province, South Africa.

Results/Discussion/Conclusion

Here we provide a detailed account of the cultural uses of *vhulivhadza* based on original ethnobotanical surveys among the *Vhavenḁa*, as well as a review of the literature. Our findings indicate that *vhulivhadza* is a "magical medicine", mostly used to induce various forms of forgetfulness, both in humans and in animals. A literature survey of the various uses reported for *L. schweinfurthii* and *L. alata*, the latter an East African species with similar root bark hairs, suggested that the use of these hairs to induce forgetfulness is confined to southern Africa. The practice of taking traditional medicine to "forget something" or "to make people forget" is quite widespread in southern Africa. We discuss the use of *vhulivhadza* and a few other plants used locally for this purpose, notably the mysterious sho-ḁā plant of the now extinct |Xam culture, *Asclepias crispa* (Apocynaceae: Asclepiadoideae) and *Galium tomentosum* (Rubiaceae).

Chemically the roots of *Lannea schweinfurthii* and other members of the genus are rich in phytochemicals, with at least some screenings for biological activity suggesting the presence of compounds that may affect the neurological system. In the absence of any comprehensive chemical or pharmaceutical analyses of

vhulivhadza itself, the claimed memory-altering activities of this material can tentatively perhaps best be explained by psychological rather than physical (chemical) effects, but a more definite scientific explanation must await further study.

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Ethnomedicinal study of ferns called “cuti cuti” in Perú

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Introduction

There is evidence about popular use of medicinal ferns for diabetes called "cuti cuti". Occasionally the common name “cuti cuti” is related to others, such as “raqui-raqui”, “doradilla”, “white cuti”, “yellow cuti cuti”, “female cuti cuti”, “male cuti cuti” or “domain grass”^{1,2,3,4}. Diabetes mellitus is an insulin-related disease, generally associated with insufficient synthesis of this or non-recognition by the target organs. In Peru, those affected increase as a result of various factors such as overweight, obesity, lack of physical activity and inappropriate lifestyles^{5,6}.

Method

Herbariums and markets were visited in the cities of Lima, Huancayo, Huaraz, Trujillo, Chiclayo, Piura, Cajamarca, Arequipa and Cusco. Ferns were collected in the field and dried for a descriptive-morphological study.

Results / Discussion / Conclusion

"Cuti cuti" is comprised of six species of ferns, which are: *Argyrochosma nivea* (Poir.) Windham, *Cheilanthes pruinata* Kaulf., *Myriopteris aurea* (Poir.) Grusz & Windham, *Myriopteris myriophylla* (Desv.) J. Sm., *Myriopteris scariosa* (Sw.) Fée y *Pellaea ternifolia* (Cav.) Link. All inhabit humid cracks of rocks, 1500 meters above sea level. "Cuti cuti" is equivalent in Spanish to "return return" or "come back come back". In regions of Peru, such as Cusco, people believe that it can restore (or bring back) the soul of one who has suffered a strong emotional impact. In addition, in Cusco and Huancayo, they are used at Christmas time to decorate Nativities. During the rainy season, they are sold while still fresh; while dry versions are used the rest of the year. For diabetes, it is prepared like a tea with five to six branches (approx. 5-6 gr.) in a liter of boiling water, then allowed to steep. This beverage may then be consumed throughout during the day. The "cuti cuti" sold in Piura and Chiclayo corresponds to *P. ternifolia*; in Trujillo, *M. myriophylla*; in Huaraz and Lima, *A. nivea*, *C. pruinata*, *M. aurea* and *M. scariosa*; in Huancayo *A. nivea*; and in Arequipa and Cusco, *A. nivea* and *C. pruinata*. The latter two varieties sold in Arequipa and Cusco are not marketed as a

diabetes treatment, though they are known to be such. The areas where "cuti cuti" are normally marketed as a diabetes treatment are the larger cities found along the coast; such as Lima, Trujillo, Chiclayo and Piura.

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Healing biospheres in ethnomedicine intangible cultural heritage proposal

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Introduction

In recent decades, Ethnomedicine, which deals with health procedures in a temporal-spatial vision, offering preventive and therapeutic strategies of different cultures in the world, is again considered an important issue in health research. From an initial historical and ethnographic interest of this discipline, it has turned in recent years into research laboratory involving biomedicine in particular pharmacology, thanks to the the World Health Organization initiatives.

In fact, the WHO Traditional Medicine Programme is a response to the needs of renewed interest in popular therapies and the identification and eventual use within National Health Services. The Declaration of Alma Ata, in the international conference on primary health care in 1978, builds the historical foundations for the official policy of the Traditional Medicine Programme (WHO,1978) thus opening the door to a dialogue between several different health systems, traditional ones and modern ones, however, putting the condition that the support given to traditional medicines and to professionals who practice and use traditional remedies, should be developed through a process which will promote those practices that are safe and effective, on the basis of adequate medical and scientific experimentation.

Method

The UNESCO Chair project “anthropology of Health, Biosphere and Healing Systems” stems from a cultural need and a heritage asset. The cultural need takes into account of contemporary trends in scientific research, aiming towards a recognition of a link between the concepts of health, environment and nursing. This approach overcomes the traditional separation between fields of knowledge - science, culture, nature - towards a new and integrated system. The concept of health and treatment methods do not have universal value: universal is the scientific quality of the survey. The long-term goal is to train a generation of multidisciplinary researchers, Western and not able to translate the second integrated sets scientific methods

of knowledge of Ethnomedical world, capable of reaching a very knowledge in different ways. This approach considers the economic and social development since the strength of the traditional community economies points.

Results/Discussion/Conclusion

The idea to candidate a Healing Biosphere as a UNESCO material and immaterial heritage starts with the evidence that half of the world population does not use western biomedicine and prefers to relate to traditional or indigenous medicine for their healthcare; many of these are related to forests. The Forest as context is usually organized between different layers: the geomorphologic traits and its climate, the biosphere, the ethnosphere and the noosphere, embedded together in a unique system where water, plants, animals, medicines, memories and spaces generate the complex dynamism of the landscape of healing. In this context the ceremonial architecture, as well as the rest of the landscape reveal a cosmologic approach to the harmonization of both individuals and communities, where the memory of sacred sites plays a crucial role.

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Study of the chemical composition of 5 batches of bank of the marine plant *Thalassia testudinum*

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Seagrasses are an important component of marine ecosystems near the coast and are an important source of secondary metabolites with important pharmacological properties. The knowledge of the seasonal behavior of the chemical composition of the plants is of vital importance in the establishment of the quality specifications of a natural product. The objective of this study was to determine the content of polyphenols, thalassiolin B and the content of metals in 5 batches of *Thalassiatestinum*. The study of the polyphenol content was carried out by the FolinCiocalteu method, the content of thalassiolin B was determined by High Performance Liquid Chromatography (HPLC) in isocratic mode using acetonitrile: water as mobile phase and the metal content was determined by Spectrometry of Atomic Absorption and Flame Photometry. The results show that between the months of study the extracts made with leaves collected in may and june showed the highest values of phenolic compounds and thalassiolin B, also allowed to demonstrate the no presence of heavy metals in the evaluated extracts. These results constitute evidence of the chemical potentialities of *Thalassiatestinum* as an active ingredient in formulations for medicinal or cosmeceutical purposes.

Palabras clave: *Thalassiatestinum*, polifenoles, thalassiolina B, metales.

Diseño de dos formulaciones de infusiones a base de plantas medicinales y frutas deshidratadas

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Introducción

Las plantas medicinales y frutas contienen entre sus componentes, vitaminas, minerales, compuestos fenólicos y antioxidantes, los cuales les proporcionan varias actividades terapéuticas. (El Sohaimy et al, 2015), (Formagio et al, 2015). En la actualidad se busca introducir productos agradables y de fácil consumo, es por esto que el objetivo de este proyecto es diseñar dos formulaciones para consumir en forma de infusión a base de plantas medicinales (*Moringa oleifera* Lam. e *Hibiscus sabdariffa* L.) y frutas deshidratadas (piña, mango, naranja).

Métodos

El estudio se inicia con la determinación de algunos parámetros físicoquímicos (pH, acidez, °Brix, humedad e índice de madurez) y la deshidratación de materias primas. Para el desarrollo de ambas formulaciones se parte de un diseño experimental completamente al azar, tomando en cuenta el porcentaje o proporción de fruta deshidratada a utilizar para las formulaciones de M. oleifera y de H. sabdariffa. Se establecieron 3 mezclas para cada formulación las cuales fueron designadas con un código 1A; 2A; 3A (moringa (1,5 g) con 5 g de piña y mango; 3 g de naranja) y 1B; 2B; 3B (flor de Jamaica (2 g) con 6,5 g de piña y mango). Para seleccionar la mejor combinación se aplicó una prueba sensorial con la ayuda de 23 jueces semientrenados, utilizando la respuesta sensorial como variable dependiente en el diseño. Se realizó un análisis de varianza y una prueba de rangos múltiples a los datos obtenidos de esta evaluación.

Resultados

Del análisis de varianza aplicado a los datos obtenidos dentro de la valoración sensorial, se logró determinar que, en el caso de las formulaciones de té de moringa, existe una diferencia estadísticamente significativa entre la media de la respuesta sensorial de los evaluadores frente a las formulaciones, con un nivel del 95.0%



de confianza, la mezcla que tuvo mayor aceptación fue la combinación 1A. Para el caso de las formulaciones de té de Jamaica también se evidenció una diferencia estadísticamente significativa entre las mezclas, resultando la de mayor aceptación la formulación 3B. A las infusiones seleccionadas de moringa y jamaica se les realizó análisis físico-químicos (acidez, pH, °Brix, humedad), además se determinó el análisis de fibra dietaria en las frutas deshidratadas de cada infusión, obteniendo 8,9% para la combinación (piña, mango, naranja) y 8,8% para la combinación (piña, mango). En las frutas deshidratadas de cada infusión, los minerales que sobresalen son: K (1,32%) y Fe (41,1 ppm mg/ Kg) para la mezcla de piña, mango, naranja, y; K (1,14%) y Na (42,3 ppm mg/ Kg) para la mezcla de piña, mango. La cuantificación de fenoles totales se realizó mediante el reactivo de Folin Ciocalteu, obteniendo los siguientes resultados: infusión de moringa 72,70 mg/200 mL, frutas 67,35 mg/200 mL; infusión de Jamaica 75,81 mg/200 mL, frutas 71,93 mg/dosis de fruta. La determinación de la capacidad secuestradora de radicales libres frente al 2,2-difenil-1-picrilhidrazilo (DPPH) se calculó por medio de la concentración inhibitoria media (CI₅₀) de cada muestra, obteniendo los siguientes valores: infusión de moringa CI₅₀= 1,302 mg/ mL, frutas CI₅₀= 3,396 mg/ mL; infusión de Jamaica CI₅₀= 0,85 mg/ mL, frutas CI₅₀= 1,988 mg/ mL.

Conclusiones

Los resultados obtenidos demuestran que la infusión que mostró una mayor capacidad secuestradora de radicales libres fue la combinación de H. sabdariffa con piña y mango, ambas deshidratadas.

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Estudio etnobotánico de plantas medicinales en un consejo popular de la provincia Granma

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Introducción

La Organización Mundial de la Salud (OMS) ha estimado que más del 80% de la población mundial utiliza la medicina tradicional para satisfacer sus necesidades de atención primaria de salud. Gran parte de los tratamientos tradicionales implica el uso de extractos de plantas o sus principios activos.

Método

Con el fin de caracterizar el uso popular de las plantas medicinales en el consejo popular Dos Ríos del municipio Jiguaní, Granma, se realizó un estudio etnobotánico entre los meses de febrero a mayo del 2016. Se entrevistaron a 205 personas, de ambos sexos y mayores de 20 años.

Resultados / Discusión / Conclusión

Como resultado se reportaron un total de 46 familias, agrupados en 90 géneros y 101 especies; las familias más representadas fueron Lamiaceae, Asteraceae y Fabaceae. Las partes de las plantas más utilizadas fueron las hojas, la vía de administración fue la oral y la forma de preparación la decocción. Se obtuvieron 67 usos diferentes, principalmente: anticatarral, problemas digestivos y para los riñones. De las 20 plantas más reportadas, se calcularon los indicadores etnobotánicos cuantitativos para la cual la menta, orégano, salvia y la manzanilla fueron los que obtuvieron un Nivel de Uso Significativo superior al 20%; el mayor Índice de Fidelidad según el mayor número de citas lo obtuvo la menta para problemas digestivos con un 69,05%. La planta que mostró un Índice de Consenso entre los Informantes igual a 1 fue el llantén para el tratamiento de la amigdalitis. Se reportaron 10 nuevos usos, el más citado es para los nervios, asociado a la especie *Plectranthus neochilus* Schltr.(Meprobamato)

Study ethnobotany of the medicinal plants used by the population of Pinar del Río

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Abstract

Ethnobotany studies constitute the base for the development of programs guided to obtain the maximum knowledge on the use of the traditional medicine, the enrichment of the cultural wealth and the best use in the patrimony of the medicinal plants. The survey methods are the most widespread, if one keeps in mind that selected sample embraces the most remarkable experts in the area herbalist`s, quack doctors, traditionalist, adults of more age. It is carried out an inventory starting from a survey made to residents from different towns in the county, according to the informations gathered about the use of the medicinal plants in the area, that which constitutes a valuable contribution to evaluate the potential of our medicinal flora in their diversity. Leaving of the preparation of the 3er year-old students from the subject The Agricultural Education in the Current Cuban School that is imparted in the Pedagogical School “Tania laGuerrillera”; the same ones carry out the labor component in the different primary schools of the county, from which were carried out the different surveys. The results were presented, which allowed the traditional use of 110 medicinal plants that are used in 29 different affections to settle down. They are related the scientific names of each species, their grouping in family, their representativeness taxonomic, as well as the properties that are attributed them in the study area. These results are presented in a multimedia which allows to visualize the plants, the parts of the same ones that are used and the places where they are in the different communities; as well as videos of the surveys and interviews to specialists. It allows to enrich the primary teacher's professionalism and therefore the school becomes an promoters from a conception of sustainable development.

Key words: biological diversity, agricultural education for the sustainable development, environmental education, medicinal plants.

Anti-*Helicobacter pylori* properties of three species of Mexican “quelites”

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Introduction

The “quelites” are comestible green plants that include both, young and tender herbs, as well as some flowers, inflorescences and stem tips of perennials. Even though quelites have been consumed in Mexico since pre-Hispanic times, nowadays they are considered as neglected and underutilized species despite their nutritional value and ethnic importance¹. In addition to their well-known use as food, some of these species have been traditionally recognized for having beneficial health properties, as is the case of their usage for the treatment of some gastrointestinal ailments.

Helicobacter pylori is the main etiological agent of gastritis, peptic ulcer and gastric cancer. It is estimated that more than half the world's population is infected by this Gram- negative bacteria that successfully infects the human stomach by using unique strategies of colonization².

In order to incentivize the reinsertion of these plants in the daily diet by demonstrating an added health-promoting value, in this work, the anti-*H. pylori* properties of three representative species of quelites, *Anoda cristata* (Alache), *Cnidioscolus aconitifolius* (Chaya) and *Crotalaria pumila* (Chepil), as well as the gastroprotective effect of the mucilage from Alache were tested.

Method

Alache, Chaya, and Chepil were collected at Tepetlixpa, Estado de México, Mérida, Yucatán, and Ocotlán de Morelos, Oaxaca, respectively. Aqueous and dichloromethane-methanol extracts were obtained from the three species of quelites. Also, the mucilages from Alache and Chepil were isolated. All samples were assayed *in vitro* against *H. pylori* growth [by determining the Minimal Inhibitory concentration (MIC)] and upon two of its colonization factors (adherence and urease activity). The gastroprotective effect of the mucilage extracted from the Alache was tested in an ethanol-induced gastric ulcer model in mice.

Results/Discussion/Conclusion

Dichloromethane-methanol extracts from Chaya, Chepil and Alache, exert the best inhibitory effect on bacterial growth, with MIC's of 62.5, 125 and 250 $\mu\text{g/mL}$, respectively. All the extracts and mucilages inhibit bacterial adhesion by 30 to 50%. None of them have effect on urease activity³. The mucilage of the Alache possesses a good gastroprotective activity, >50%.

Considering the antibacterial effect, the anti-adhesive properties, and the gastroprotective potential found in the studied species, these plants could not only represent a promising source for a complementary therapy, but also, their regular consumption may play an important key role in reducing bacterial colonization.

This work provides new information about the anti-*H. pylori* potential of three edible quelites, and provides the basis for further studies to establish whether their consumption in a daily diet could have an impact on the prevention and/or control of *H. pylori* associated diseases.

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The referring centre for phytoterapy “cerfit”: an italian experience with medical Cannabis

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Introduction

The Referring Center for Phytotherapy of Tuscany Region named CERFIT is set in the Careggi University Hospital in Florence (Italy), it conducts intensive research, with participation in drug research, clinical and epidemiological analysis of the products within some University Departments. It is a research group cross professionally, integrated and complementary, consisting of physicians, pharmacologists, pharmacists, nutritionists, herbalists, acupuncturists, chemists, biologists and epidemiologists, with the collaboration of experts in anthropology, ethnomedicine, nursing, midwifery and sanitary economy. Since 2006 in Italy medical doctors can prescribe masterly preparations, set up by the pharmacist in pharmacies, containing Dronabinol or herbal active substance based on medical cannabis (i.e. the active substance obtained from the inflorescences of *Cannabis sativa*: they are dried and ground, administered per os -in the form of decoction or oil- or per inhalation with a special vaporizer). It is farmed with the authorization of a National organism for cannabis. Tuscany was the first Region in Italy (May, 2012) to legislate on the subject of medicinal Cannabis. Since then, Cannabis is fully entitled to care opportunities offered by the Tuscan public health service, and can be prescribed for therapeutic purposes by physicians.

Method

The first evidences on the use of Cannabis for therapeutic purposes are dated from the third millennium BC in China, as described in the text *Nei-Ching* written between 2700 and 2600. The plant was prescribed to treat diarrhea, bronchitis, migraine, insomnia, in appetite and nervous disorders. In India the use of cannabis indica in the second millennium BC is evidenced in the sacred texts of the Vedas in which the demon of the drunkenness *Vide-Vadat*, is symbolized by the hemp which also had the function of promoting ecstasy. In India the cannabis indica is called *Bhang* and takes on an important religious significance as it is



the favorite plant of the god Shiva; his followers use it as a source of mystical inspiration. In 2016, Italy started a national production of cannabis for medical use at the Military Pharmaceutical Chemical Plant of Florence (SCFM), thanks to the collaboration between the Ministry of Health and the Ministry of Defense, in order to guarantee access to such therapies at adequate and safe costs. At the end of 2017, the collaboration between the Region and the Military Pharmaceutical Chemical Plant has intensified and expanded, both in terms of Cannabis and orphan drugs production for the treatment of rare diseases.

Results/Discussion/Conclusion

Currently the Tuscany Region is planning the establishment of an Agency for Cannabis, a technical-scientific body aimed at the coordination and planning of production, extraction, therapeutic use and research on Cannabis. Through its activity, it could provide information and tools to support regional planning (potentially useful also at a national level). Essential objectives: assistance, continuity of care, fairness of access and outcome, concerning the medicinal use of Cannabis, promotion of correct information and training of health personnel, and activation of specific phytovigilance process

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Bixin and Norbixin deposition in different tissues of healthy rats

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Introduction

The color in many foods determines preference and acceptability, there are artificial and natural dyes used in the food, pharmaceutical and cosmetic industry. The achiote *Bixa orellana* L. is the most commercialized tropical botanical tree species native to tropical America, distributed from Mexico to Brazil and Argentina, also found in the Caribbean. In Panama it has been registered throughout the country (Source: Herbarium of the University of Panama). This shrub produces seeds called also achiote, the seeds are sources of an excellent dye that is widely used in the artisanal and food industry¹. On the other hand, extracts from different parts of the achiote are used in traditional medicine and have demonstrated effects against diabetes, among other diseases². Considering its wide consumption and that there are few studies that speak of its accumulation³, our objective was to evaluate the accumulation of bixin and norbixin in different tissues of healthy rats fed daily with these carotenoids.

Method

Healthy male SD (Sprague Dawley) rats (n = 8) of approximately 350g, were fed for 30 days with an extract of bixin and norbixin at a concentration of 60 mg of carotenoid/kg of weight/day. Weight and intake were monitored daily. At the end of the study the deposition of bixin and norbixin were evaluated in the different tissues³. Bixin was extracted from achiote seeds (*Bixa orellana*), with acetone until they had no color. The ketone extracts were collected, filtered and stored at 4°C for quantification and use. A portion of the ketone extract was dried and saponified with 5% KOH Methanol for 30 minutes, then neutralized with HCl and extracted with a mixture of ethyl ether: hexane (1: 1). In the quantification of total carotenoids, in tissues and extracts, it was performed in a spectrophotometer at 450 nm. The saponification reaction was followed by HPLC equipped with a UV/Visible photodiode matrix detector.

Results and Discussion

A greater accumulation was observed in the spleen, liver and lung, in these three tissues norbixin accumulated in greater proportion (1.1; 0.80 and 0.40 μ g/g tissue, respectively). In adipose tissue and in the

heart, bixin accumulated more than norbixin (**Figure 1**). This may be due to its less polar character, due of the methyl group. In plasma, the concentration of both carotenoids was very low, (data is not shown).

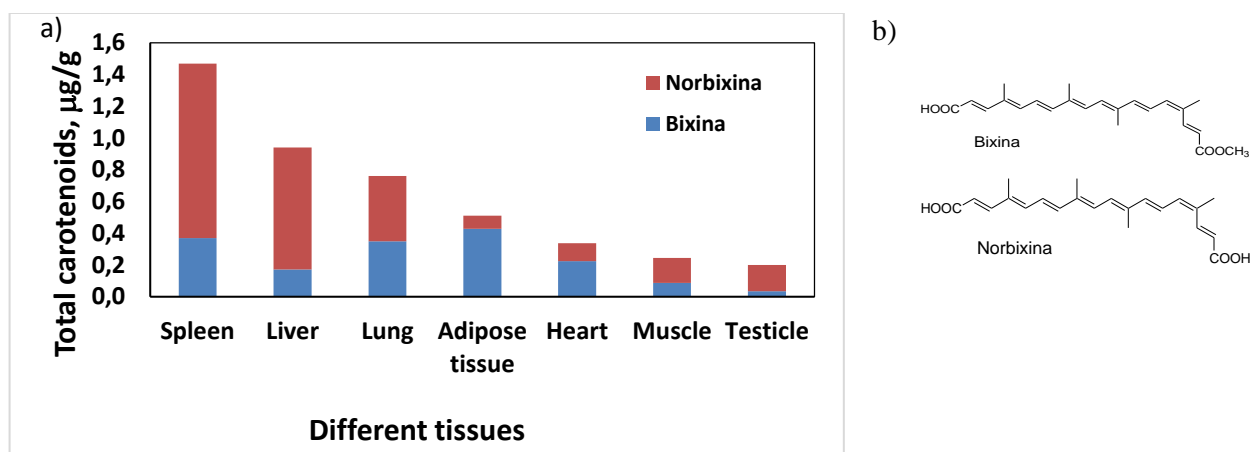


Figure 1. Deposition of bixin and norbixin in different rat tissues. a) Carotenoid content in different tissues and b) Structure of Bixin and Norbixin.

Conclusion

The total carotenoid in spleen and liver was higher for animals that consumed norbixin. On the other hand, in adipose tissue and heart, the total carotenoids were higher in the group of animals that followed the bixin diet.

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Antiproliferative and genotoxic potential of flavonoids isolated from leaves of *Chromolaena tacotana* R.M. King & H. Rob on MDA-MB-231 and PC-3 cancer cell lines

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Introduction

The use of medicinal plants has provided a starting point for the development of new drugs that contribute to the prevention and treatment of cancer. Flavonoids have antioxidant and or antiproliferative properties that depend on its structure-activity relationship (Batra & Sharma, 2013). *Chromolaena tacotana* is a plant widely distributed in Colombia, and characterized by its content of flavonoids, but their anticancer activity is not well known.

Method

Four flavonoids were isolated from leaves of *Chromolaena tacotana* from dichloromethane extract by column chromatography (Si gel G 60-200 microns 4 x 60cm) eluted with CHCl₃, mixtures of CHCl₃: MeOH, and MeOH and they were identified as: 5,3'-dihydroxi-7,4-dimetoxiflavanol (F1), 3',4'-dihydroxi-5,7-dimetoxiflavanona (F2), 4'-hidroxi-5,7-dimetoxiflavanona (F3) and 4-hidroxi-5,7-dimetoxiflavanonol (F4) (Rodríguez et al., 2018). Cytotoxicity was analyzed by employing MTT assay on PC-3 and MDA-MB-231 cancer cell lines, and the genotoxicity was assessed by using comet assay (Dent et al., 2007). Microtubules and nuclear morphology changes were evaluated by using of fluorescence microscopy.

Results / Discussion / Conclusion

Results indicated that the cytotoxicity of flavonoids on MDA-MB-231 cells was very low compared with the effect on PC3 cells. F2 and F3 were the flavonoids with the highest antiproliferative effect on PC3 cells, with IC₅₀ values of 40.24 µg / mL and 49.17 µg / mL respectively. The analysis of the effect on cellular morphology showed that F4 affects the microtubules and nuclei of MRC5, healthy fibroblasts used as control, while F1, F2 and F3 altered microtubules and nuclei of PC-3 and MDA-MB- 231 cancer cells. Data analysis of genotoxicity showed that flavonoids F1 and F2 induced minimal damage to the DNA of the analyzed cells lower than 10%, whereas flavonoid F3 altered the integrity of DNA, with values between 20

and 30% on PC3 and MDA-MB-231 and less than 19% in MRC5, while taxol a positive control used for genotoxicity showed a percentage of DNA damage greater than 80% in all cell lines including healthy. In conclusion, it was demonstrated that F2 and F3 showed the highest antiproliferative and genotoxic effects on prostate cancer cells, contributing to the study of the antineoplastic potential of the genus *Chromolaena*

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Evaluación del efecto de (-)-epicatequina en la expresión de perilipinas en un modelo murino de esteatosis hepática no alcohólica inducida por dieta

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Introducción

La esteatosis hepática no alcohólica (EHNA) se caracteriza por el acúmulo de grasa en forma de gotas lipídicas, representando el 5 al 10% del peso del hígado y es concomitante con el incremento de triglicéridos (TG)¹. Los tratamientos se enfocan en cambios en el estilo de vida y no existe tratamiento farmacológico que sea del todo efectivo². Por ello, surge la necesidad de nuevas alternativas que ayuden al tratamiento de la EHNA sin efectos adversos y de bajo costo, como los flavonoides, por ejemplo la (-)-epicatequina (EC) que ha mostrado tener efectos sobre la regulación del metabolismo de lípidos y disminución del riesgo de enfermedades cardiovasculares y metabólicas³. Por lo anterior, el objetivo fue evaluar la expresión de las perilipinas en un modelo murino de esteatosis hepática no alcohólica inducida por dieta alta en colesterol y colato (DP).

Método

Ratones C57B6/L de 20 a 25g, fueron distribuidos aleatoriamente en 3 grupos (n=24 en cada uno): (1) dieta normal y agua, (2) DP y fructosa 30% y (3) DP + fructosa + EC por 9 semanas. La dosis de EC fue de 1mg/kg/día vía intragástrica. Se registró el peso semanal. Se realizó la eutanasia a los 7, 21, 35 y 60 días. Se determinó el perfil bioquímico y enzimas hepáticas en suero. El hígado se pesó y analizó macro y microscópicamente, y se cuantificó los TG acumulados. Finalmente se evaluó la expresión relativa de las perilipinas (Plin1, Plin2, Plin5 y Plin3) y proteínas asociadas al metabolismo de lípidos (CD36, Adiponectina, UCP1 y UCP2).

Resultados y discusión

El tratamiento con EC redujo la ganancia de peso (14%) y evitó el incremento de los TG, LDL-c y TG/HDL (29, 38 y 49% respectivamente) así como de la glucosa (18%); además, la actividad de las enzimas hepáticas

AST ALT y ALP se mantuvo por debajo del grupo que recibió solo la dieta alta en colesterol y colato (33%, 14% y 30% menos, respectivamente). El peso del hígado fue 27% más bajo que el grupo DP, y el contenido de TG fue 21% menor. Las características macroscópicas del hígado mostraron menor acúmulo de grasa en presencia de EC y el análisis microscópico mostró menor "balonización" del hepatocito, gotas lipídicas pequeñas, así como, menor acúmulo de colágeno e infiltración de células inflamatorias. Finalmente, se encontró que algunas proteínas relacionadas al metabolismo de lípidos como CD36 fueron más bajas, mientras que adiponectina, UCP1 y UCP2 se incrementaron. Respecto a las perilipinas: Plin 1 no se expresa en el hígado, Plin 2 disminuyó, plin 5 y 3 no se modificaron. Los resultados son similares a lo reportado en estudios con epigallocatequina-3-galato (EGCG) presente en el té verde, utilizada en modelos murinos de EHNA inducida por dieta alta en grasa. EGCG es un isómero de EC que contiene ácido gálico, haciéndolo potencialmente tóxico, por lo que el flavanol libre EC es una mejor opción ya que no hay reportes de toxicidad y es un producto catalogado como seguro (GRAS: Generally Recognized As Safe) por la FDA.

Conclusión

EC muestra mejora en el perfil bioquímico, así como en el acúmulo de TG en el hígado y sus características macroscópicas y microscópicas. Pero además presentó efecto sobre la expresión de perilipinas, particularmente Plin 3 manteniéndola y Plin 2 disminuyéndola. Por lo tanto, EC debe ser considerada como un posible abordaje terapéutico para la EHNA ya que evita la progresión del daño hepático y metabólico inducido por dietas altas en grasa.

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Computational study of interaction of cannabidiol analogs with CB₁ and CB₂ receptors

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Introduction

Cannabis sativa is currently used as an element of recreational use at a general level; however, it has been attributed different pharmacological properties with important clinical applications and have been involved complicated treatment diseases (Lotan et al. 2014; Press, Knupp, and Chapman 2015; Kafil et al. 2017). In the phytochemical studies carried out over the years, a number of secondary metabolites other than tetrahydrocannabinol (THC) have been identified, such as cannabiol, cannabidiol and analogues, with highly promising biological activity (Zuardi et al. 2009; Burstein 2015). Furthermore, the role of these structures with CB₁ and CB₂ receptors have been identified as the main targets of the *endo*-cannabinoid system, as well as their interaction with other receptors related to pain, obesity, depression and immunosuppression (Huang, Chen, and Zhang 2016; Rossi et al. 2018). To evaluate the activity of cannabidiol and analogues by docking molecular and molecular dynamics for refining.

Method

Initially, the molecules (ligands) were selected by bioinformatics search in databases, then reverse docking was carried out to identify targets with greater affinity; subsequently, with the crystallographic reports of the CB₁ and CB₂ receptor, evidenced crystalline structure in the databases. Afterward, each receptor and its ligands were optimized to finally perform the docking molecular. The most stable ligand-receptor complexes were simulated by molecular dynamics to refine and evaluate the activation-free energy. CB₁ and CB₂ receptors were selected after carrying out the reverse docking.

Results/Discussion/Conclusion

From the docking molecular it was obtained that the most stable molecules with the CB₁ receptor were BHQ, AC1MIYKR, AC1L4XIK, ChEMBL68869, ChEMBL32300 and AC1Q7B7D; while the molecules with the highest score on the CB₂ receptor were ChEMBL32300, THC, BDBM50181832, LS-61175, dronabinol and cannabicitran. The molecular dynamics studies allowed to refine the ligand-receptor complexes, evidencing



the presence of interactions with amino acid residues such as LEU 359, PHE 379, CYS 386, VAL 196, PHE 268 and PHE 102 of CB₁ and CB₂ with their ligands. We identified new cannabidiol analogue metabolites with high activity in cannabinoid receptors indistinctly. Finally, the receptor and ligand complexes were refined by means of molecular modeling showing that the interactions considered of most importance were of a hydrophobic nature. These metabolites presented are promising for pharmacological activity related to both CB₁ and CB₂ receptor.

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Antihyperglycemic activity of the leaves from *Annona diversifolia* safford. and farnesol

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Introduction

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels resulting from insulin resistance or inadequate insulin secretion. In the world, DM is one of the most frequent noncontagious diseases that affect more than 371 million people. *A. diversifolia* is known in Mexico as “ilama and papause,” the fruits of this plant are used as food; as part of traditional medicine in some regions from Mexico people use their leaves as anticonvulsant, analgesic, and anti-inflammatory; at present, it is used to control hyperglycemia in people with DM.

Method

Bioassay-guided fractionation of the ethanol extract of the leaves of *A. diversifolia* (EELAd) was performed on alloxan-induced Type 2 diabetic and normoglycemic (NM) mice. Oral glucose tolerance test (OGTT), oral sucrose tolerance test (OSTT), and oral lactose tolerance test (OLTT) were performed in fast NM mice (FNM).

Results/Discussion/Conclusion

The EELAd, CHCl₃ fraction, and farnesol induced a significant reduction of postprandial hyperglycemia in acute and subchronic tests using AITD mice. When EELAd, CHCl₃ fraction, and farnesol were tested on NM in subchronic assay did not affect glyceic levels. In the case of acute test on NM, only CHCl₃ fraction induced a hypoglycemic effect at 2 h after the treatment. OLTT and OSTT showed that the EELAd, CHCl₃ fraction, and farnesol induced a significant reduction of hyperglycemia levels in FNM at 2 h after a lactose or sucrose load comparable to acarbose. In the case of OGTT was observed similar effect in FNM mice at 2 h after a glucose load comparable to canagliflozin. Conclusion: The EELAd and farnesol induced a significant reduction of postprandial hyperglycemia on AITD mice in acute and subchronic assays. Our results suggest that the control of postprandial hyperglycemia may be mediated by the regulation of absorption of glucose and inhibition of disaccharide digestion such as sucrose and lactose. Finally, the results explained the use of *A. diversifolia* in Mexican traditional medicine as an antihyperglycemic agent.



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Effects of *Clinopodium tomentosum* (Kunth) Govaerts ethanolic extract on Porcine Aortic Endothelial Cells (pAECs) stability

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Primary culture of porcine aortic endothelial cells (pAECs) have been widely used in different in vitro assays to evaluate the behavior of endothelium against external agents¹. *Clinopodium tomentosum* (Kunth) Govaerts is an Ecuadorian medicinal plant commonly used in the traditional medicine to treat cough, toothache and headache².

This research aimed to assess the effects of a *Clinopodium tomentosum* ethanolic extract (CTEE) on pAECs stability. Quantitative analysis using HPLC-DAD was conducted to determinate the relative amounts of different phenolic acids and flavonoids. Cells stability in vitro were carry out by MTT assay, scratch test and capillary-like tube formation.

The phytochemical analysis showed a high quantity of different phenolic acids and flavonoids. Rosmarinic acid and hesperidin were the major active compounds. Different concentrations (1 – 200 µg/mL) of CTEE did not affect pAECs viability. Moreover, CTEE increased cell migration by about 59% and induced a complete capillary like network increasing the number of master junctions in a concentration dependent manner. In conclusion, our results demonstrated the pro-angiogenetic properties of the *Clinopodium tomentosum* ethanolic extract.

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A preliminary study on *Couropita guianensis* bark: chemical characterization and biological effect on placental mesenchymal stem cells

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Introduction

Couropita guianensis is a medicinal plant of the Lecythidaceae family and it has a wide use among Amazonian Natives. This plant showed many activities, as: antibacterial, antiparasitic, antifungal, antidiarrheal, neuropharmacological, immunomodulatory, antioxidant, antitumor, antipyretic, analgesic and antiinflammatory.

The aim of the study was to characterize the profile of secondary metabolites and the metal content of the aqueous and ethanolic extracts of *C. guianensis* bark. Two sets of analysis are organized to typify the main metabolites/ metal of the bark and focusing successively on the antioxidant activity of its extracts after administration on Placental Mesenchymal Stem Cells too.

Method

The first set includes: qualitative colorimetric tests, quantitative tests (Folin-Ciocalteu, Fast Blue BB, Aluminium chloride), Inductive Coupling Plasma Atomic Emission Spectroscopy (ICP-AES) analysis. A second set of analysis includes 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, MTT assay and MTT assay in induced oxidative stress on Placental Mesenchymal Stem Cells to test the antioxidant activity and the absence of cytotoxicity on this cell line.

Results/Discussion/Conclusion

The presence of triterpenoids, reducing sugars, alkaloids, saponins, phenols and anthroquinones in the extracts of *C. guianensis* was demonstrated. Flavonoid content was evaluated by Aluminium chloride method and resulted 0.173-0.223 mg/ml Quercetin equivalents. Phenolic content was evaluated with FBBBMethod and Folin-Ciocalteu Method and resulted to be 4.774-2.790 mg/ml Gallic acid equivalent and 1.780-1.010 mg/ml Gallic acid equivalent respectively. Eighteen metals were quantified using ICP-AES. The results of antioxidant activity evaluated by DPPH assay showed values of 300-437 $\mu\text{g/ml}$ in terms of IC50. Citotoxic activity measured by MTT assay at 24-48-72h from the administration of the extracts, at different concentration, on Placental Mesenchymal Stem Cells demonstrated that 0.1% concentration is the most active. These data improve the knowledge on the less investigated bark of *Couroupita guianensis*, particularly the study provides the first metal quantification and a citotoxicity evaluation of the aqueous extract on Placental Mesenchymal Stem Cell, observing a lack of citotoxicity at concentration of 0.1% in induced oxidative stress too.

Efecto cicatrizante del aceite esencial de *Mansoa alliacea* (Ajos giros), en heridas por ovariectomía en *Canis lupus familiaris*

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Introducción

El conocimiento del uso de las plantas medicinales es ancestral, heredado de generación en generación en diferentes civilizaciones y culturas, como tradición, para diferentes fines.

Yambay (2013), afirma que una de las problemáticas que se presenta en la actualidad es la cicatrización de las heridas; cualquiera que sea la vía de cicatrización, debido a que se dan las mismas fases, y cada una requiere de la anterior para que pueda darse.

En este contexto se vienen realizando investigaciones que brinden alternativas no sintéticas para enfrentar el proceso de cicatrización, a fin de reducir el tiempo de cicatrización, pero a la vez que sea de fácil uso y no representen un mayor gasto; en ese sentido se tiene el uso de plantas medicinales, dentro de las que está la *Mansoa alliacea*, “ajos giros”, que de acuerdo a estudios etnobotánicos contiene metabolitos que pueden favorecer el proceso de cicatrización.

La presente investigación se propuso como objetivo evaluar la eficacia del aceite esencial de *Mansoa alliacea* (Ajos giros), obtenido por el método destilación por arrastre, como cicatrizante en heridas por ovariectomía en *Canis lupus familiaris* (perras); y determinar el tiempo de reducción y el de desinflamación de la herida.

Método

Se evaluaron las heridas producidas por ovariectomía en 10 perras de diferentes edades y pesos, las cuales se separaron en dos grupos (Grupo A= aceite esencial de *Mansoa alliacea* y grupo B= Rifamicina 1%), cada grupo conformado por 5 perras. El efecto cicatrizante se evaluó a través de histogramas y el análisis de proporciones.

Resultados/Discusión/Conclusión



La cicatrización en el grupo A tratado con aceite esencial de *Mansoa alliacea*, se determinó el día 13 con 40% de cicatrización completa. El grupo B tratado con Rifamicina 1%, inicia la cicatrización el día 14 con un 20% de cicatrización completa. La cicatrización completa del 100% de perras para el grupo A se presentó en el día 15 y para el grupo B el 100% de cicatrización se presentó el día 17. La reducción de la cicatriz fue del 60% para el grupo tratado con *Mansoa alliacea* y solo el 20% redujo su cicatriz en el grupo tratado con Rifamicina 1%.

Al análisis de proporciones, para determinar la significancia del efecto cicatrizante del aceite esencial de *Mansoa alliacea*, se encontró que hay significancia en ambos grupos. La rápida aparición de la costra en la mayoría de animales tratados con aceite esencial de *Mansoa alliacea* se le puede atribuir a que dentro de su composición fitoquímica cuenta con vitamina C (Calero, 2010); además Sánchez y González (2016) reportan que la vitamina C, es necesaria para la producción de colágeno, lo cual favorece la formación de la costra y rápida recuperación de la herida. Esto demuestra que el aceite esencial de *Mansoa alliacea* tiene efecto cicatrizante, respaldando lo reportado por Gonzales (2013), donde menciona que esta planta tiene propiedades vulnerarias, cicatrizantes, concluyendo que el aceite esencial de *Mansoa alliacea* es eficaz como cicatrizante en heridas por ovariectomía en *Canis lupus familiaris*.

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Quantification of metals in *Solanum tuberosum* (papa) and *Tropeolum tuberosum* (cubio) in crop of the municipalities of sibaté and villapinzón

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Introduction

WHO recognizes the fundamental role of food safety in public health; The diseases transmitted by the intake of food with some contaminants, are an important burden for the economy of a country. A large number of people get sick and many die from consuming foods containing harmful substances that accumulate and become toxic. There is evidence that environmental pollution caused by industrial development dumping is the main source of deterioration of water masses adjacent to crops. This study was carried out in crops near the Bogotá River, where water is sometimes used for irrigation; food absorbs contaminants, by the root, becoming a threat to human health.

Objectives

Quantify the accumulation of metals (cobalt, copper, chromium, nickel, manganese, lead and zinc) of *Solanum tuberosum* (potato) and *Tropeolum tuberosum* (cubio) in the Sibaté and Villapinzón crops

Methodology

Acid digestion was performed in the samples collected with the subsequent evaluation of heavy metals by atomic absorption.

Results and Conclusions

High levels of some metals were found in potatoes and cubes.

ppm	Zn	Ni	Pb	Mn	Fe	Cu	Cr	Co
Papa Villapinzón	2.82	0.09	0.05	0.07	11.64	0.78	0.02	0.09
Papa Sibaté	4.64	0.02	0.00	0.00	10.61	1.66	0.00	0.10

Cubio Villapinzón	7.78	0.07	0.08	0.11	29.50	0.60	1.91	0.12
Cubio Sibaté	1.80	0.04	0.03	0.00	39.15	0.34	0.03	0.08
Suelo papa Villapinzón	64.28	1.86	13.29	0.77	0.78	12.95	7.77	7.14
Suelo papa Sibaté	59.61	1.98	13.36	0.83	0.75	17.27	5.43	4.64
Suelo cubio Villapinzón	62.49	1.97	13.64	0.40	0.32	12.41	6.89	6.84
Suelo cubio Sibaté	39.55	2.24	8.49	0.06	1.17	27.74	3.01	4.76

Comparing with respect to resolution number 2115 of Jun 22, 2007 Colombia, where the concentrations of the elements that have a recognized adverse effect on human health are in intervals of: Copper Cu 1.0, Total Chromium Cr 0.05, Ni Nickel 0.02, Lead Pb 0.01, Iron Total Fe 0.3, Manganese Mn 0.1, Zinc Zn 3. And normal concentrations in soils (ppm): Zn 25-200, Ni 2-100, Pb 10-150, Cu 2-60, it is clear to note from the high levels found in some elements a public health problem.

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Preclinical study of the anti-inflammatory activity of the ethnolic extract of the leaves of *Manihot esculenta crantz* (yuca) in an experimental model of acute inflammation

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Background

The anti-inflammatory activity has awakened, in the last years, a great scientific interest in the pharmacological area, mainly by virtue of the potential capacity of certain compounds to interfere in the inflammatory process. The leaves of *Manihot esculenta* (yuca) have been used around the world and through time for this purpose. Objective: To evaluate the anti-inflammatory activity of the ethanolic extract of *Manihot esculenta crantz* (EEME) leaves in rats. Methods: An experimental, transversal, prospective and analytical study was carried out. The study population was 60 albino rats *sp. Rattus norvegicus*, distributed in 4 groups of 10 individuals each. A homogeneous solution of ethanolic extract of *Manihot esculenta crantz* leaves was administered via intraperitoneal (IP) injection. Carrageenan was used as an inflammatory inducer, administered intradermally, for the measurement of plantar edema, it was used the Digital Water Plethysmometer (LE7500). The dose of administration to the groups was: negative control (CN), positive control (CP) betamethasone 4mg / Kg, treatment group 1 (GT1): EEME: 2 mg / kg and treatment group 2 (GT2): EEME: 4 mg / kg. The one-way ANOVA test and the Tukey's post hoc test were used for comparisons between the groups. Results: In 37.67% of the GT2, a reduction of the edema was observed at 3 hours after the administration of the EEME ($p < 0.05$). It also shows that in both groups of administration of EEME there is a tendency to reduce plantar edema with values close to significance. Conclusions: The ethanolic extract of *Manihot esculenta* from the 4mg / kg dose demonstrates its anti-inflammatory activity by significantly reducing plantar edema.

Keywords: *Manihot esculenta crantz*, inflammation, edema.

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Anticancer evaluation and acute toxicity of the leaves from *Annona muricata* L.

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Introduction

In Mexico, the use of medicinal plants is currently a common practice as a therapeutic alternative for different diseases, including cancer (Aguilar et al., 1994; OMS, 2004). Cancer is the leading cause of mortality worldwide, in this sense there is a lot of work on medicinal plants associated with the search of anticancer compounds (Loraine and Mendoza-Espinoza, 2010). *Annona muricata* L. also known as soursop, graviola and guanabana is a member of the Annonaceae family with a long history of traditional use, being cancer one of that. Numerous investigations on this plant demonstrate that *Annona muricata* L. have several activities, including anticonvulsant, anti-arthritic, antiparasitic, antimalarial, hepatoprotective, antidiabetic and anticancer activities. In this sense, *Annona muricata* L. is a plant with high potential as a source for the isolation of anticancer compounds (Torres et al., 2013).

Method

The collection of plant species was conducted two areas of the state of Guerrero (Acapulco y Tecpán de Galeana) bimonthly; we obtained six samples by each zone. The acute toxicity (DL₅₀) of the extracts was carried out using the guideline 423 of the OCDE (OCDE), the products were administered orally. For the anticancer activity, the breast cancer model were developed on Balb/c mice inoculated with 4T1 cell line (*Mus musculus*, mouse), after the cell inoculation, the animals were treated with the extract during eight days, 28 days after the treatments, the animals were sacrificed to obtain the breast cancer tumor and to compare the weight of them with the controls (animals inoculated with 4T1 cell line without treatment and animals inoculated with 4T1 cell line with Doxorubicin treatment).

Results/Discussion/Conclusion

The DL_{50} were calculated based on the mortality of the mice and this value were able to allocate the doses to work on the model of breast cancer. Comparing the tumor weight of the extracts, the extract with the best anticancer activity were collected in Acapulco in December, showing the best effect at a dose of 20 mg/kg.

In accordance with the results obtained on this study, it is important to select carefully the samples of the leaves of *Annona muricata* L. considering the area of cultivation and season, because these determine its anticancer activity. These test results support use of the leaves from *Annona muricata* L. as anticancer agent in Mexican traditional medicine

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Efectos biológicos de los derivados del núcleo 4-oxo-tiocromano sobre *Leishmania sp*

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Introducción

La leishmaniasis es una enfermedad parasitaria que afecta 1.3 millones de personas cada año en 98 países de la región tropical y sub tropical [1],[2], ocasionando un grave problema de salud pública agravada por la crisis de los medicamentos actuales evidenciado en la manifestación de graves efectos secundarios, mecanismo de resistencia y altos precios [3]. La directora general de la organización mundial de la salud Margaret Chan ha alertado sobre esto “Nunca hemos tenido un arsenal para el tratamiento de enfermedades tan sofisticado como el actual, sin embargo, la cura para estas continúa alejándose. Esto es inaceptable” [4] por esta razón es necesario el Desarrollo de nuevas alternativas terapéuticas económicas y seguras.

En atención a la mencionada crisis, en nuestro laboratorio se han desarrollado algunas moléculas del tipo cromano y tiocromanos que presentan altas actividades y selectividades contra esta patología [5], [6],[7]. Sin embargo, en este camino también se han obtenido moléculas con un mal perfil farmacológico, pero, con una alta drogabilidad, por lo cual, en este trabajo se ha desarrollado la optimización molecular del ácido 4-oxotiocromano-2-carboxílico.

Método

Las reacciones químicas usadas para la funcionalización del compuesto inicial fueron planteadas siguiendo la estrategia de incorporar algunos de los sustituyentes más frecuentes presente en los medicamentos que se comercializan actualmente. [8] Mediante reacciones del tipo “one pot” o ben, enmarcadas en el concepto de las reacciones click.

Los ensayos de actividad biológica *in vitro* fueron realizados sobre amastigotes intracelulares de *L. panamensis* y la citotoxicidad se determinó sobre células musculares humanas de línea celular U-937.

Los análisis *in silico* para el análisis de interacción ligando proteína sobre la enzima tripanotion reductasa (TR) fueron desarrollados con el protocolo open source SwissDock [9] y los análisis de estructura actividad (SAR), fueron calculados en la plataforma datawarriror [10].

Resultados

La metodología empleada permitió obtener 20 derivados estructurales del ácido 4-oxotiocromano-2-carboxílico, cuyas modificaciones químicas que incluyen centros quirales permitieron aumentar 14,6 veces su actividad anti-leishmanicida inicial, alcanzando el rango micromolar medio 28, 38 μM . Un análisis SAR de dicha optimización, permitió encontrar posiciones sensibles para la actividad y efectos enantioméricos, así como relaciones de la flexibilidad de los sustituyentes con el aumento de la actividad. Adicionalmente, en un esfuerzo para entender el posible mecanismo de acción de estas sustancias se desarrolló un estudio de docking molecular sobre la enzima tripanotion reductasa (TR) cuyos valores de la función de pose son congruentes con los valores de actividad biológica, lo cual, valida las herramientas de predicción, usadas para proponer una siguiente generación de compuestos con una estrategia basada en el blanco terapéutico.

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Actividad antioxidante del germinado de cuatro variedades de *Chenopodium quinoa* Willd. “quinua” del Perú

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Introducción

Chenopodium quinoa Willd., es una especie alimenticia nativa de la región andina de Sudamérica, su semilla posee compuestos con propiedades promotoras de la salud como ácidos fenólicos, fitoesteroles y flavonoides¹. Pasko et al. (2008), determinaron el contenido de los compuestos fenólicos presentes en la semilla y en el germinado². Brend et al. (2012), evaluaron el contenido de fenoles totales y la actividad antioxidante de las variedades amarilla y roja³. En la presente investigación, se propuso determinar el porcentaje de germinación, el contenido de fenoles totales y flavonoides; y el porcentaje de actividad antioxidante del germinado de semillas de cuatro variedades de *Chenopodium quinoa* Willd. “quinua”.

Método

Se utilizó 100 g de semillas de las variedades negra “ccoito”, roja “pasankalla”, blanca “choclito” y amarilla “marangani”. La obtención del germinado se realizó según Xue et al (2016), se determinó al altura (cm) y el porcentaje de germinación⁴. 5 g del germinado desecado y pulverizado fueron extraídos con etanol 96% y concentrados a sequedad en un evaporador rotatorio. Los fenoles totales se determinaron por el método de Singleton y col., utilizando el reactivo de Folin – Ciocalteu, expresados como µg equivalentes de ácido gálico/g de extracto (µg GAE/g de extracto)¹. Los flavonoides se determinaron por el método de Dini y col., utilizando el cloruro de aluminio, expresados como µg equivalentes de quercetina/g de extracto (µg QE/g de extracto)¹ y la actividad antioxidante se evaluó por el método de secuestro del radical libre 1,1 – difenil – picril – hidrazilo (DPPH), expresado como porcentaje de captación⁵. Las diferencias entre las medias de los ensayos se determinaron mediante el ANVA al 95% utilizando el SPSS versión 21.

Resultados/Discusión/Conclusión

La variedad blanca alcanzó mayor altura (3,0 cm) y mayor porcentaje de germinación (95%); mientras que la variedad roja alcanzó menor altura (1,8 cm) y menor porcentaje de germinación (40%). La variedad blanca

mostró mayor contenido de fenoles totales (59,67 μg GAE/g de extracto) y flavonoides (54,63 μg QE/g de extracto); mientras que, la variedad Amarilla mostró menor contenido de fenoles totales (28,00 μg GAE/g de extracto) y flavonoides (14,50 μg QE/g de extracto) respectivamente ($p < 0,05$). La variedad negra mostró mayor porcentaje de actividad secuestradora del radical libre DPPH (66,58%), seguida de la variedad roja (58,56%); mientras que las variedades blanca (53,21%) y amarilla (56,28%), mostraron menor actividad secuestradora ($p < 0,05$). A pesar que las variedades blanca y amarilla muestran mayor contenido de fenoles totales y flavonoides, sin embargo, las variedades negra y roja tienen mayor contenido de ácidos fenólicos libres que proporción mayor actividad secuestradora del radical DPPH⁶. Se concluye que el germinado de la variedad blanca de la semilla de *Chenopodium quinoa* Willd. alcanzó mayor altura y mayor porcentaje de germinación, mayor contenido de fenoles totales y flavonoides totales, mientras que el germinado de la variedad negra mostró mayor actividad antioxidante, seguida de la variedad roja.

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Evaluation of the antifungal activity of ethyl acetate and n-butanol extracts from *Cladobotryum virescens*

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Introduction

The negative impact of phytopathogenic fungi is a major threat to food security worldwide. However, they are a source of secondary metabolites with diverse biological activities for use in medicine and agriculture (Feng et al., 2003). Thus, the extracts of medium and high polarity obtained from the culture broth and the mycelia of several species of the genus *Cladobotryum* present antifungal activity against various phytopathogenic fungi. The extracts obtained from the extraction with different organic solvents report that the species *Cladobotryum virescens* presents positive activity against phytopathogenic fungi such as the species of *Cladosporium spp* (Ortiz et al., 2012). According to these interesting results, we proposed to evaluate the *in vitro* antifungal activity of the ethyl acetate and n-butanol extracts obtained from the culture broth of *Cladobotryum virescens* against other phytopathogens (*Botrytis Cinerea*, *Phytophthora infestans* and *Septoria tritici*) that affect agricultural production.

Method

The strain of *Cladobotryum virescens* was selected from the Collection of Pure Crops of the Institute of Fundamental Research in Tropical Agriculture " Alexander von Humboldt " and was identified by the curator Dr. Rafael Castañeda. It was subjected to a fermentation process for 15 days at room temperature in oats media. Subsequently, it was filtered to separate the mycelia from the culture broth, which was subjected to a liquid-liquid extraction with ethyl acetate and n-butanol. The extracts obtained were concentrated to dryness and the n-butanol extract was chromatographed on a DIAION HP 20 column. Water, methanol, acetone, ethyl acetate and acetone in an acidic medium were used as eluents obtaining 5 new fractions. The extracts and the fractions were tested in a 96-well microtiter plate assay against three phytopathogenic fungi *Botrytis Cinerea*, *Phytophthora infestans* and *Septoria tritici* according to the protocol established by the Action Committee of Fungicide Resistance with slight modifications (Stammler et al., 2011). The solvent DMSO



was used as a negative control and the commercially used fungicides Terbinafine and Epoxiconazole (from Merck) served as reference compounds. Seven days after inoculation, the pathogen growth was evaluated by measurements of the optical density (OD) at $\lambda = 405$ nm with a Tecan GENios Pro microplate reader (5 measurements per well using multiple reads in a 3x 3 square). Each experiment was carried out in triplicate. The results were analyzed with SigmaPlot 12.0 software.

Results/Discussion/Conclusion

The Liquid Column Chromatography using DIAION HP 20 resin and the use of different solvents as eluents allowed us to separate sugars, lipophilic compounds, carboxylic acids and other secondary metabolites according to the solvent used. The extracts and the fractions evaluated did not inhibit the growth of the pathogen *Botrytis Cinerea*. However, the ethyl acetate extract was the most active at all concentrations (125, 42, 14, 5 and 1.5 ppm) against the pathogens *Phytophthora infestans* and *Septoria tritici* reaching an inhibition of pathogen growth greater than 60%. The results suggest that the AcOEt extract is an important source of secondary metabolites with antifungal activity.

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Pharmacognostic evaluation and estimation of the content of polyphenols and flavonoids of *Moringa oleifera* cultivated in Cuba

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Abstract

Introduction: *Moringa oleifera* belongs to the Moringaceae family, it is native to the Southern Himalayas, with tropical and subtropical climate. The plant has secondary metabolites such as: Polyphenols and Flavonoids that give it its biological activity. **Objective:** To know the behavior of the secondary metabolites Polyphenols and Flavonoids, as well as the Protein content and the Moisture variable of dried *Moringa oleifera* leaves cultivated in Cuba. **Materials and Methods:** The plant material was dried at controlled temperature and humidity. The screening was performed by the classical methodology of solvent polarity fractionation. The content of Polyphenols was carried out by colorimetric techniques described in the British Pharmacopoeia, using Galic Acid and Flavonoids as the standard described by Woisk, using Quercetin as a standard. While the determination of protein and moisture was performed by near infrared spectroscopy. **Results:** The presence of: oils/fats, alkaloids, triterpenes/steroids, catechins, reducing sugars, flavonoids and phenols/tannins were observed. The concentration of Polyphenols was found between 38.63 - 91.56 mg equivalent of gallic acid/g plant material and for Flavonoids concentrations between 163.62 - 213.96 mg equivalents of quercetin/g plant material. Protein content showed values between 20%-34% and humidity was $\leq 12\%$. **Conclusions:** The study showed that the analyzed samples are within the limits of acceptance reported to be used as a Nutraceutical.

Key word: *Moringa*, Polyphenols and Flavonoids

Antibacterial sensitivity study of *Moringa oleifera* extract against strains of *Staphylococcus aureus*

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Summary

Staphylococcus aureus (*S.aureus*) is a pathogenic microorganism responsible for nosocomial infections, present in sepsis of surgical wound, burns, pneumonia associated with ventilation, among other effects. In recent years, *S.aureus* strains resistant to multiple drugs have been identified antimicrobials which makes it necessary to search for new therapeutic alternatives to combat affectations caused by this microorganism. The *Moringa* tree is native to the India and has anti-inflammatory, antimicrobial, antioxidant, anti-cancer, cardiovascular, hepatoprotective, antiulcer and diuretic activities.

Objective: The objective of the present study was to evaluate the antibacterial sensitivity of a 70% alcoholic extract of dried leaves of *Moringa oleifera* against 3 strains of *Staphylococcus aureus* from clinical isolates with resistance to multiple antibiotics.

Materials and Methods: Antimicrobial sensitivity was determined by the microdilution method according to the National Standard Clinical Laboratory Committee (NCCLS) to determine the Minimum Bactericidal Concentration (CMB). The microorganisms were adjusted to 0.5 Mac Farland scale. Double dilutions of the extracts from 100 mg / ml to 0.78 mg / ml were prepared.

Results: The 70% alcoholic extract showed antimicrobial activity against the bacterial strains studied, with CMB 3.1 mg / mL.

Conclusion: The 70% alcoholic extract of *Moringa oleifera* had a good antibacterial sensitivity against *S.aureus* strains and could be used as a source of new formulations that are used to treat infections caused by this infectious agent.



Evaluation of the hypoglycemic effect of dry leaf capsules of *Moringa oleifera* cultivated in Cuba

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Abstract

Introduction: *Moringa oleifera* is one of the best known and widely distributed species of the Moringaceae family. Virtually all parts of the tree have diverse applications, on which there are testaments since ancient times. *Moringa* leaves have hypoglycemic activity, among other biological activities. **Objective:** To demonstrate the hypoglycemic potential of capsules from the hydroalcoholic extract of dried leaves of *Moringa oleifera* of the Criolla variety cultivated in Cuba. **Materials and Methods:** The test was performed on albinos male mice, randomly distributed in 4 experimental groups; G1 control and groups G2, G3 and G4 were induced to streptozotocin hyperglycemia and G2 subsequently was treated orally with glibenclamide (5mg/mL) while G3 and G4 at doses of 1 and 2 *Moringa* capsules of 500mg / kg of mouse weight respectively. The oral glucose tolerance test was performed using the GOD-PAP method, the rest of the tests were performed according to the kit instructions (Glucose liquicolor kit). BIOCHEMICAL SYSTEM supplied by Human Co., Germany, the results were expressed in mg/dl. **Results:** The treatment with *Moringa* capsules was much better to repeated dose and indicated the hypoglycemic effect on the diabetic condition. **Conclusions:** The study showed that *Moringa* capsules after 2 months of administration had an hypoglycemic effect which was equivalent to the effect of glibenclamide that is a drug used in the treatment of diabetes.

Key word: *Moringa*, hypoglycemic activity.



Evaluation of antiviral activity of extracts from dry leaves of *Moringa oleífera* cultivated in Cuba

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Summary

Introduction: Herpes simple virus (HSV-1) causes some of the most common viral infections humans. Currently, scientific development has led to interest in medicinal plants and natural bioactive substances for the development of antiviral substances. Objective: The aim of this study was to investigate the *invitro* [antiviral activity](#) of different concentrations of aqueous and hydroalcoholic extracts from three varieties of *Moringaoleiferaplant* against reference strains of Herpes simplex virus type 1 and type2 in [Vero cells](#) *in vitro* condition. Materials and Methods: The cytotoxic concentration (CC₅₀) as well as the Efective concentration (EC₅₀) of the extracts was determined against 100 Infectious Dose in Cell Culture (DICC50) of viruses. The effect was determined by the MTT colorimetric method. Results: It was observed that the aqueous extracts of the varieties studied did not present toxicity from 500 µg / mL while the alcoholics were not toxic at the concentration of 250 µg / mL. None of the extracts were able to inhibit the replication of the viruses studied.

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Different triterpene mixtures present anti-inflammatory properties and reduce their toxicity when they are in a plant extract

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Introduction

Obesity-associated inflammation is a risk factor associated with multiples diseases. New anti-inflammatory treatments are targeting this adipose tissue inflammation (Reilly SM. et al., 2017). Ursolic acid (UA) and Oleanolic acid (OA) are natural triterpene compounds with anti-inflammatory and anti-oxidant properties that suggest these molecules could have a promising role in the treatment of inflammatory disorders (Dae Yun Seo et al., 2018). We have identified UA, OA and Ursolic acid lactone (UAL) as the main molecules (78%) in an *E. tereticornis* extract and shown the mixture of these three different triterpenes has a potent effect of reducing pro-inflammatory cytokine levels in adipose tissue and other immunometabolic abnormalities generated in adipose tissue cells (Guillén A., et al. 2015; Susana Ceballos, et al. 2018). Our aim is to compare the effects of the triterpenes mixture present in the natural extract with the same total amount of different combinations of the molecules outside the extract on macrophage cell viability, oxidative stress and expression of pro-inflammatory genes and proteins.

Method

A crude methanolic extract (OBE100) from leaves of Eu was partitioned with ethyl acetate, concentrated and purified by Sephadex LH-20. UA and OA were purchased from Sigma-Aldrich (St. Louis, MO) and UAL was purified by Sephadex column and preparative chromatography from OBE100. Activated murine macrophage cell line J774A.1 was used to evaluate OBE100 and different triterpenes (UA, OA and UAL) mixtures anti-inflammatory activity. Cell line viability was determined using a MTT assay, oxidative burst was quantified by flow cytometry using Dihydrorhodamine 123 (DHR), pro-inflammatory gene expression levels were detected by real-time RT-PCR and protein expression were detected by CBA.

Results / Discussion / Conclusion

Triterpenes mixtures with lower concentration of UAL are more toxic than OBE100 extract with the same total amount of triterpenes. Only the treatment with OBE100 reduced the oxidative burst in activated macrophages. All the triterpenes mixtures have some anti-inflammatory activity but no mixture improves the reduction of pro-inflammatory genes and proteins expression observed by OBE100 treatment. These results suggest that triterpenes present in *E. tereticornis* extract have anti-inflammatory properties, independently how we mixed them, but the combination of these triterpenes with other minor molecules, as they are present in the vegetal extract, have an additive or synergistic effect that improves them, reducing their toxicity and presenting antioxidant properties.

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Antioxidant hydrolysates of different whey protein concentrates

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Introduction

One of the most important components of whey is protein due to its chemical, nutritional and biological properties. These proteins are an important source of peptides with biological activity; however, due to its high dilution in whey, it is important to implement techniques to isolate and concentrate whey proteins before hydrolytic process. For this reason, the objective of this study was to evaluate the enzymatic hydrolysis in different whey protein concentrates to obtain hydrolysates with antioxidant properties.

Methodology

Three methods for recovery of whey proteins were evaluated: thermal precipitation (TP), salt precipitation (SP) and ultrafiltration concentration (UC); subsequently, these concentrates were subjected to hydrolysis with three different proteases (alcalase, chymotrypsin, flavourzyme). The soluble protein content, free amino acids, degree of hydrolysis and antioxidant activity (ABTS radical scavenging activity, determination of reducing power and iron-chelating activity) to each hydrolysate were determined.

Results / Discussion / Conclusion

Concerning the protein concentrates obtained, the total protein content was 53, 75 and 49% (w/w), with a yield percentage of 43, 28 and 46%, in the products obtained by TP, SP and UC, respectively. A decrease in the protein content in the SP and UF concentrates, and an increase in the free amino acid content in all concentrates after 24 h of hydrolysis were observed. The SP and UC concentrates showed a higher degree of hydrolysis with alcalase and flavourzyme. The antioxidant properties were superior after hydrolysis of the concentrates with different enzymes. The ABTS radical scavenging and iron-chelating activities were greater in the SP and UC concentrates when they were hydrolyzed with alcalase and chymotrypsin. Additionally, the reducing power was greater in all flavourzyme hydrolysates. The increase in the antioxidant activity of the hydrolysates may be related to the presence of bioactive peptides with specific structural characteristics in



the different hydrolysates. In conclusion, the production of whey protein hydrolysates with antioxidant properties can be an interesting alternative as a strategy for the valorization of whey that allows its use as a source of compounds with health effects to be used in the development of added-value food products, generating a significant contribution in the area of functional foods. The composition of these hydrolysates can be affected by the protein concentration method employed and the enzyme specificity used during the hydrolytic process.

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Quantification by hptlc-densitometry of *Salvia purpurea* (Cav) Lamiaceae

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Introduction

Salvia purpurea (Cav) Lamiaceae is a medicinal plant used in infusion for its properties as a toothache relief and stomach pains in the state of Oaxaca¹ and Chiapas², Mexico. The sage genus mainly synthesized triterpenes and sterols like ursolic acid, oleanolic and β -sitosterol³. A previous study revealed the antinociceptive effect from extracts of *Salvia purpurea* in murine models. In this sense, we evaluated by HPTLC-densitometry metabolites in active extracts of this plant.

Method

The aerial part of *S. purpurea* was dried at room temperature and then, triturated. The dried material was subjected to 3 consecutive extractions by 24 hrs each one in acetone and methanol, the solvents were evaporated by distillation under reduce pressure. For the aqueous extract, a decoction and then, was lyophilized. The determination and quantification of terpenoids and flavonoids in extracts of *Salvia purpurea* was by HPTLC-densitometry. The application of the extracts and standards was in chromatographic thin layer in band forms with ATS4 (CAMAG). The plates were eluated in its corresponding mobile phases and scanned with Scanner3 (CAMAG) using specific wavelengths for each metabolite or standard. The essays were evaluated in triplicate. The metabolites were quantified using calibrations curves for each metabolite in software WinCATS (CAMAG).

Results/Discussion/Conclusion

The analysis by HPTLC showed that the acetone and methanol extracts have more metabolites of the kind terpenoid, with 12 and 9 respectively, we identified stigmasterol, β -amirin and mix of acid ursolic and acid oleanolic, and this mixed were the most concentrated in both extracts. On the other hand, the polar extracts showed metabolites like flavonoids, the extracts have 11 and 9 different flavonoids in methanol and aqueous

respectively. We identified quercetin and kaempferol in high concentrations in the acetone and methanol extracts and in a little concentration in the aqueous extract. Likewise, the rutin was identified only in the aqueous extract. This result showed that salvias mainly synthesized metabolites of kind terpenoids, where the acid ursolic and oleanolic are the major constituents.

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Sinergismo de la combinación del veneno del escorpión *R. Junceus* con citostáticos convencionales.

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Introducción

El veneno del escorpión endémico de Cuba, *Rhopalurus junceus* (*R.junceus*), disminuye la viabilidad de las células tumorales de origen epitelial y no tiene efecto citotóxico sobre células normales. Sin embargo, se desconoce el tipo de muerte celular inducido por la combinación de este veneno de escorpión con citostáticos convencionales. El objetivo de nuestro trabajo es determinar la potencialidad del veneno del escorpión *R.junceus* de incrementar el efecto citotóxico de los citostáticos convencionales sobre células tumorales.

Materiales y Métodos

Las células tumorales de colon murino CT26, se trataron simultáneamente a una razón constante de concentraciones iguales o inferiores a la concentración inhibitoria media (CI₅₀) del veneno del escorpión *R.junceus* y de los citostáticos doxorubicina y cisplatino, respectivamente. La viabilidad celular se determinó por el ensayo de MTT, transcurrido 72 horas de incubación y el porcentaje de células apoptóticas se determinó mediante el marcaje de AnexinaV/PI después de 24h de combinación con la CI₅₀.

Resultados

La combinación del veneno con cisplatino y doxorubicina reduce significativamente la viabilidad de la línea tumoral CT26, en comparación con los tratamientos individuales. El tipo de interacción farmacológica de dicha combinación es sinérgica a altas concentraciones. Además, el tratamiento combinado incrementa el porcentaje de células en apoptosis tardía en comparación con los tratamientos individuales. **Discusión:** Entre los tipos de tumores de mayor impacto a nivel mundial se encuentra el cáncer colorrectal (CCR) que es el tercero en incidencia y el segundo en mortalidad (Moradi y cols., 2018; Rademaker y cols., 2019). Estudios preclínicos realizados han demostrado las potencialidades antitumorales de los venenos de escorpión



(Moradi y cols., 2018). El tratamiento individual (Díaz-García y cols., 2013; Díaz-García y cols., 2017) y combinado (Yglesias-Rivera y cols., 2019) del veneno de escorpión endémico de Cuba, *R. junceus*, disminuye la viabilidad de células tumorales de origen epitelial. Este trabajo, nos acerca por primera vez al tipo de muerte celular que se induce con la combinación del veneno *R.junceus* con citostáticos convencionales; inclusive se evalúa por primera vez el efecto de la CI_{50} sobre los eventos de muerte celular.

Conclusión

El veneno de escorpión *R.junceus* en combinación con doxorrubicina y cisplatino incrementa la citotoxicidad de estos agentes quimioterapéuticos sobre células tumorales colorrectal.

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Estudio comparativo farmacognóstico y fitoquímico del genero *Prosopis* (*P. pallida* y *P. juliflora*), cultivado en Ecuador

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Resumen

El algarrobo es una especie del genero *Prosopis* perteneciente a la clasificación de las leguminosas. Si bien la literatura (Prokopiuk 2000) reporta varias especies de algarrobo como la africana, flexuosa, alba, entre otras; en Ecuador se reconocen dos especies de algarrobo tropical: *Prosopis pallida* y *Prosopis juliflora* (Aguilera, 2014). Ambas especies, aparentemente, se ubican en las provincias costeras y en la frontera sur del país.

Al algarrobo se le han atribuido diferentes aplicaciones, destacando entre ellas sus propiedades alimenticias y farmacológicas, además de su uso forrajero y maderero. Sin embargo, no existe suficiente información acerca de la composición química y particularmente el contenido mineral de *Prosopis pallida* de origen ecuatoriano que permita caracterizar la especie y así diferenciarla de otras variedades de algarrobo presentes en nuestro país.

Por tratarse de una especie vegetal de gran interés por ser considerada como un recurso nativo del escenario rural ecuatoriano, el presente trabajo lleva a cabo una caracterización farmacognóstica y fitoquímica de la especie *P. pallida* cultivada en la provincia de Santa Elena, en comparación de los mismos parámetros analizados en la hoja, corteza y vaina de *P. juliflora* cultivada en la misma provincia.

Los ensayos realizados incluyeron la aplicación de métodos clásicos para la medición morfológica, farmacognóstica y fitoquímica. La determinación del contenido mineral se realizó mediante ICP-OES, en muestras tratadas previamente bajo condiciones optimizadas por digestión ácida de los 3 tipos de órganos de las especies vegetales, con valores satisfactorios de precisión (n=5).

Los resultados fitoquímicos presentan correspondencia con otras especies del genero *Prosopis*, destacándose la identificación de metabolitos secundarios tales como; alcaloides, compuestos fenólicos y azúcares. La composición mineral evidencia minerales mayoritarios como calcio y potasio en ambas especies, con valores



entre 629,30 - 3198,86 mg Ca/100g de muestra y entre 996,52 - 1877,38 mg K/100g de muestra, en los órganos ensayados por separado, Otros metales abundantes fueron magnesio, sodio y fósforo, con valores superiores a 100 mg; mientras que elementos minoritarios con valores inferiores a 40 mg/100g de muestra se reportan para bario, manganeso, zinc, boro y estroncio.

Los resultados en general permiten concluir que las especies estudiadas, poseen características similares, siendo una de las pocas diferencias ciertos atributos físicos tales como el peso, y tamaño de las vainas; así como el contenido de ciertos microelementos como hierro, aluminio que se presentaron en mayor concentración en la *P. juliflora*, y cobre que solo fue detectado en *P. pallida*, existiendo la debida correlación en la presencia de los minerales identificados. Este trabajo proporciona información relevante para la caracterización de las especies ecuatorianas.

Palabras clave: algarrobo, *Prosopis*, metales, ICP

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Pharmacological evaluation of *Plectranthus scutellarioides* (Lamiaceae)

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Introduction

The Mazatec culture, has one of the traditional medicines of prehispanic origin, where natural medicines were easily obtained from exuberant local biota, highlighting the "hierba de la pastora" (*Salvia divinorum*) for its psychoactive properties. After the European invasion, the mazatecos initiate the use of other non-native species, such as *Plectranthus scutellarioides*, substitute of the "hierba de la pastora". Also, in San José Vista Hermosa, Oaxaca, *P. scurellarioides*, known as "morado", is used to treat hemorrhages and dysentery¹. In other cultures, this species has a long history of therapeutic use to cure angina, asthma, bronchitis, digestive problems, epilepsy, insomnia, urticaria and scorpion stings. Chemical studies report that *P. scurellarioides* synthesizes essential oils, sterols, triterpenoids, alkaloids, saponins, flavonoids and tannins². According to the background, in this work the anxiolytic and antinociceptive activity of the organic extracts of this plant was evaluated, as well as the identification of terpenoids and flavonoids in the active extracts.

Method

The aerial part of *P. scutellarioides* was collected in San José Vista Hermosa, the plant material, was dried, crushed and subjected to three successive extractions, using solvents of increasing polarity (n-hexane, ethyl acetate and methanol). For the pharmacological evaluation, male CD1 mice were used, which were administered by esophageal route (p.o.) the dose of 300 mg/kg of each extracts, in addition, a control group was included, which was given a solution saline. To determine the anxiolytic effect, the extracts were evaluated in three models: open field, hole board and open arms, using clonazepam (0.5 mg / kg, p.o.) as a reference drug. Pain assessment was performed using the "Writhing" test, using as ketorolac control (10 mg /

kg, p.o) and as a nociceptive agent, 1% acetic acid, administered intraperitoneally. The nociceptive behavior assessed was the frequency of abdominal stretches performed every 5 minutes for 30 minutes. Thin layer chromatography was performed for the identification of terpenes and flavonoids present in the plant.

Results/Discussion/Conclusion

The three extracts in the evaluated dose had significant anxiolytic activity with respect to the control in the open arms test. Likewise, the experimental subjects decreased their activity in the open field test, suggesting a sedative effect. On the other hand, all extracts decrease nociceptive behavior but was not significant. The sedative-anxiolytic effect of *P. scutellarioides* may be due to the presence of terpenoids such as α -amyrin, β -amyrin, stigmasterol, oleanolic acid and ursolic acid in all extracts and the existence of the flavonoids quercetin, canferol, rutin and naringin in the ethyl acetate and methanol extracts. All compounds were identified in *P. scutellarioides*, they have already been reported with anxiolytic effects. This work corroborated the use of this plant in mazatec medicine as anxiolytic.

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Medicinal plants of Santiago Quiotepec, Oaxaca, Mexico: Ethnobotany and Antibacterial activity

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Introduction

Santiago Quiotepec, one of the oldest communities of the Tehuacán-Cuicatlán Valley has a great tradition using medicinal plants. The aim of this study was to make an inventory of the medicinal species used by the inhabitants of Santiago Quiotepec and evaluate the antibacterial activity.

Method

Sixty semi-structured interviews were carried out with informants from the community of Santiago Quiotepec. Intentional sampling was used to select the informants, providing personal data and information referring to the use of medicinal plants. Botanical techniques of collection and herborization of the species mentioned during the interviews were also carried out. Of the information obtained from the interviews, frequency of appearance was determined and the diseases were categorized according to the symptom referred. Fifteen species that have been used to cure illnesses of possible bacterial origin (gastrointestinal, respiratory, ophthalmological, gynecological) and which don't have studies of their biology activities were selected to evaluate their antibacterial activity. Three extracts of different polarity (hexanic, acetyl and methanolic) of each of the species were obtained and evaluated on 19 strains of bacteria of medical importance according to the diffusion method in agar and the active extracts were determined the Minimum Inhibitory Concentration (MIC).

Results/Discussion/Conclusion

A total of 60 informants were interviewed, 66 species of plants used in the treatment of different diseases were mentioned, which belong to 34 families and 62 genera. *Amphipterygium adstringens*, *Erethia latifolia* and *Aloe vera* were the most frequently mentioned species. A total of 34 diseases were mentioned in the interviews, with 16% gastrointestinal diseases were the most common (stomachache, diarrhea, vomiting), 10% inflammatory process (external and internal), 7% diabetes and 6% respiratory diseases (cough, flu). The

results obtained in the bioassays showed that the lowest values were presented in the hexane extract of *Plumbago pulchella*, with a MIC of 0.25 mg/mL over *Staphylococcus aureus* and *Staphylococcus epidermidis* as well as the hexanic extract of *Echinopterys eglandulosa* showed a MIC of 0.25 mg/mL over *Pseudomona aeruginosa*.

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Antimicrobial effect of some macro algae from the Veracruz Reef System National Park (SAV), México.

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Introduction

In recent years, algae have been subjected to various studies to corroborate the biological activities they have, however the investigations of the algal resources in Mexico are very scarce.

Method

The Ingenieros, Isla de Enmedio and Salmedina reefs, were selected to collect the material within the Veracruz Reef System National Park (SAV). The extracts were obtained by maceration using methanol and hexane. Once obtained, they were filtered and distilled under reduced pressure in a rotary evaporator (Domínguez, 1979).

The antibacterial activity of the extracts was evaluated qualitatively and quantitatively using Gram-positive and Gram-negative strains by the Kirby-Bauer method and dilution in agar. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined for those active extracts (Koneman, 1996). The antifungal activity was evaluated by the method of inhibition of radial growth (Wang & Bun, 2002).

Besides, tests were carried out to determine the main groups of secondary metabolites in the extracts. The data was analyzed using ANOVA.

Results/Discussion/Conclusion

Six species of macro algae of the three groups were collected. Only the methanolic extracts of *Dictyota cervicornis* from Isla de Enmedio was active against two Gram-positive strains and three Gram-negative

strains and *D. cervicornis* from Salmedina was active against one Gram-positive strain. The strains most susceptible to the extracts was *S. aureus* (MIC= 1.0 and 0.5 mg/mL, respectively).

Only the methanolic extract of *Padina gymnospora* showed activity on *Candida albicans*. The methanolic extracts of *D. cervicornis* from Isla de Enmedio and Salmedina were active against the same strain *Staphylococcus aureus*. The presence of phenols and glycosides were detected in the methanolic extracts of *D. cervicornis*.

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Anticancer Activity on Human Oral Cancer Cell Lines of Essential Oils from Leaves of *Piper rivinoides* Kunth (Piperaceae)

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Introduction

Many essential oils (EO) show a great variety of biological properties, such as cytotoxic, antibacterial, anti-inflammatory and leishmanicidal. *Piper rivinoides* Kunth belongs to the Piperaceae family, is native to Brazil and has wide distribution from North to South. This plant has been used in rituals of African bases religion and it is popularly known as betis-branco. The therapeutic potential of *P. rivinoides* EO has been described in the literature, with emphasis on the activities against *Mycobacterium tuberculosis* and *Leishmania* spp (Flora do Brasil, 2020; Da Silva *et al.*, 2017; Bernuci *et al.*, 2016). Oral squamous cell carcinoma (OSCC) is one of the 10 most common types of cancer. Plants of the genus *Piper* are used in traditional medicine in the treatment of cancer and they present a diversity of phytochemicals with cytotoxic potential. This work aimed to investigate *in vitro* antitumor of the EO of two samples of *P. rivinoides* collected in two different fragments of Atlantic Forest from metropolitan regions of the State of Rio de Janeiro, Brazil.

Method

Fresh leaves (150g) of *Piper rivinoides* collected at Serra da Tiririca (Niterói, RJ) and Tijuca Forest (Rio de Janeiro, RJ) were submitted to hydrodistillation for two hours in a modified Clevenger-type apparatus to obtain the EO. Compounds identification and quantification were done by Gas Chromatography coupled to Mass Spectrometry (GC-MS) and GC coupled to the Flame Ionization Detector (GC-FID). The retention index was calculated from a homologous series of hydrocarbons (C₈-C₂₆, Sigma-Aldrich) to achieve compounds identification. The EO were tested for cell viability (MTT test), using carboplatin as a positive



control. SCC9 cell lines were used for all assays. From the dose-response curves of the EO and non-linear regression, the concentration values required for the 50% inhibition of the cells (IC_{50}) (GraphPadPrism 5) were calculated. The selectivity index (IS) was calculated as: IC_{50} of normal cells / IC_{50} of tumor cells, an $IS > 2$ indicates that the compound is selective.

Results/Discussion/Conclusion

It was possible to identify 12 compounds in both EO that showed to be very similar. The major identified compounds were the monoterpenes α -pinene, β -pinene and limonene. The EO from fresh leaves of *P. rivinoides* from Tijuca Forest showed an $IC_{50} = 78.29 \pm 0.04$ and from Serra da Tiririca showed an $IC_{50} = 78.00 \pm 0.04$, therefore very similar. The positive control carboplatin presented an $IC_{50} = 318.4 \pm 0.09$. It can be concluded that the EO of *P. rivinoides* were active against the SCC9 cells line, since they had a significant inhibitory effect on the viability of oral squamous cells. The same response in SCC9 cells line can be explained by the chemical composition of EO which is very similar and can be attributed to the monoterpenes.

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Levels of Pb, Cd and As in seaweeds *Kappaphycus alvarezii* and *Kappaphycus striatus* from Palau Bidong (Malaysia)

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Introduction

The red seaweed, *Kappaphycus alvarezii* and *Kappaphycus striatus* are cultivated extensively as a source of carrageenan which have been used for decades in food applications and are generally regarded as safe.

In order to satisfy the growing demand of raw materials due to the demographic increase of world population, described by FAO estimates, the researchers have identified the seaweeds as a possible source of integration in animal feeding. There are different studies in literature on the chemical-nutritional characteristics and the benefits of their use, but further studies are needed to identify any toxicological risks related to their use [3].

As a consequence, a monitoring of heavy metals concentrations, in seaweeds is necessary from the point of view of contamination. In light of this, the aims of the present study were to quantify the total concentrations Cd, Pb and As in two species of seaweed *K. alvarezii* and *K. striatus* collected in Palau Bidong, Malaysia and to assess the health risk related to animal consumption.

Method

K. alvarezii and *K. striatus* were collected in Palau Bidong, Malaysia, cultivated in off-shore. After sampling, the algae were dried and stored until analysis. Samples of seaweed were homogenized by mixer and subsequently were digested in ultrapure 65% HNO₃ and H₂O₂ in a microwave digestion system. Concentrations of Cd, Pb and As were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) technique using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT. The calibration curve and two blanks were run during each set of analyses to check the purity of the chemicals. Reference material (CRM DORM-4, NRC, Canada) were also included for quality control. All the values of the reference materials were within certified limits. Instrumental detection limits,

expressed as wet weight (w.w.) and determined following the protocol described by Perkin Elmer ICP application study number 57 [1].

Results/Discussion/Conclusion

Heavy metals concentration in seaweed *Kappaphycus alvarezii* and *Kappaphycus striatus* are summarized in Table 1. Data are expressed as the mean concentration of metals with associated standard deviations for all analyzed samples.

	<i>K. alvarezii</i>		<i>K. striatus</i>	
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>
As	0,097	0,003	0,082	0,016
Cd	0,006	0,001	0,011	0,000
Pb	<LOQ	-	0,004	0,003

Table 1. Mean concentration and standard deviations (SD) of trace elements (mg/kg dry weight) in seaweed *K. alvarezii* and *K. striatus*. LOQ (limit of quantification).

Compared to maximum levels of heavy metals set by EU Commission Cd, Pb and As levels in seaweed always resulted lower than the maximum values established for feed materials. In fact, the EU regulation establishes the following MLs of heavy metals content in mg/kg (ppm) relative to a feed materials: the Cd, Pb and As MLs in the feed materials are 2.0, 10.0 and 2.0 mg/kg respectively [2].

Overall, our results indicate that the risk of exposure to heavy metals from consumption of seaweeds is relatively low and in compliance with EU regulations. Although, further studies on a greater number of samples are needed on metals and other pollutants. Concerning Pb, Cd and As concentrations, these preliminary results support the possibility to use these seaweed species for animal feed with no additional hazards.

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Diseño de una nueva suspensión farmacéutica utilizando microemulsiones como vehículo para la incorporación de ibuprofeno

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Introducción

El aceite de chía es un compuesto obtenido de la semilla de una planta herbácea denominada *Salvia hispánica* reconocida por tener actividades antiinflamatorias, hepatoprotectora y antidiabéticas, utilizada en la fitoterapia del cáncer y artritis reumatoides¹. El ibuprofeno es un fármaco antiinflamatorio no esterooidal, indicado en el tratamiento de la artritis reumatoide, a pesar de bastante utilizado, este fármaco presenta algunas características que limitan su uso, como la baja solubilidad en agua y como consecuencia una biodisponibilidad limitada. En este sentido, las microemulsiones (ME) han ganado mucha atención como sistemas transportadores de fármacos. Éstas son clasificadas como sistemas transparentes, termodinámicamente estables, capaces de incorporar principios activos de distinta naturaleza. Una característica importante que posee las ME es la mejora en la solubilización de fármacos lipofílicos, lo que puede mejorar su biodisponibilidad. Con lo mencionado, se propone desarrollar una nueva suspensión base utilizando las microemulsiones como vehículo para la incorporación del ibuprofeno.

Métodos

Se elaboró un diagrama de fases con diferentes proporciones de aceite (chía), agua y una mezcla binaria de tensoactivos (Tween80 y Tween20). El estudio de solubilidad del fármaco en las ME se llevó a cabo agregando sucesivas dosis de 0,01g del fármaco hasta total saturación, las muestras seleccionadas fueron caracterizadas por microscopía de luz polarizada, calorimetría diferencial de barrido (DSC), empleando el rango de temperatura de 25°C a -80°C y de -80°C hasta 120°C. En la estabilidad de los sistemas se estudió durante seis meses atendiendo los parámetros de pH, aspecto y estructura. Se desarrolló una suspensión base con vehículo a base de ME para la incorporación del ibuprofeno, la cual se realizó un análisis del perfil de liberación *in vitro* con un equipo automatizado adaptado, que contiene seis células de Franz modificadas con una membrana sintética de acetato de celulosa 0,45 µm.



Resultados/Discusión/Conclusión

Los resultados mostraron un sistema transparente homogéneo y fluido, estas características se mantuvieron durante seis meses, lo que sugiere una estabilidad de las microemulsiones en este período. Se observó un campo oscuro para la microscopia de luz polarizada, el cual sugiere la formación de microemulsiones, se incorporó 250mg de ibuprofeno en el sistema. Los valores de pH fueron 4,90 y 4,87 en el tiempo cero para los sistemas sin y con fármaco respectivamente, ambas muestras presentaron cambio de pH con el tiempo, valores bajos de pH pueden sugerir que la matriz del sistema está más organizada. El análisis de DSC indicó una interacción del ibuprofeno con las microemulsiones, ya que no se observa el pico de fusión del fármaco. Ambos sistemas (ME y suspensión/ME) aumentaron la liberación del fármaco comparadas al ibuprofeno libre, sin embargo, se observa una mejor actividad para la suspensión con ME/ibuprofeno, se comparada a la ME/ibuprofeno. Con lo expuesto, se sugiere que las ME son sistemas prometedores vehículos en el desarrollo de suspensiones para la incorporación del ibuprofeno.

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Evaluación de la permeación y retención percutánea del extracto acuoso de *Curcuma longa* incorporado a cristales líquidos

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Introducción

Curcuma longa es una especie de origen asiático, ampliamente reconocida por sus propiedades culinarias, cosméticas y medicinales. Diversos estudios han demostrado su potente actividad antiinflamatoria, anticancerígena y antimicrobiana, atribuidas principalmente a los compuestos presentes en sus rizomas, curcumina (70-77%), demetoxicurcumina (18-20%) y bisdemetoxicurcumina (5-10%). Desafortunadamente altas dosis del extracto alcohólico y/o acuoso, a través de la administración oral, puede generar efectos secundarios tales como: gastritis, obstrucción de vías biliares e hipersensibilidad.

La ciencia ha avanzado en la búsqueda de nuevas alternativas tecnológicas que mejoran las limitaciones de determinados principios activos. En este sentido, los cristales líquidos (CL), gracias a sus propiedades anfífilas, han demostrado eficacia en la liberación de fármacos por las diferentes vías de administración. Estos sistemas isotrópicos, son capaces de evitar la degradación química de los fármacos, disminuir los efectos secundarios y aumentar la biodisponibilidad. En vista de esto, se ha propuesto evaluar la permeación y retención percutánea del extracto acuoso de *Curcuma longa* incorporado a cristales líquidos.

Métodos

El diagrama de fases ternario fue construido empleando diferentes proporciones de agua (W), aceite (O) y tensoactivo (T). Para las muestras seleccionadas, se incorporaron dosis sucesivas de 0,01 mL del extracto acuoso de *Curcuma longa* hasta la saturación de los sistemas. La caracterización se llevó a cabo mediante microscopía de luz polarizada. El estudio de estabilidad fue realizado durante seis meses, para evaluar cambios en el pH, estructuras microscópicas (fase cúbica, lamelar y hexagonal) o variaciones en las características macroscópicas (color y aspecto). Para el DSC se utilizó 4,0 mg de las muestras, las cuales fueron llevadas a distintas temperaturas: 25 °C a -80 °C y de -80°C hasta 120°C. El estudio de permeación y retención percutánea *in vitro* fue realizado por un periodo de 24 horas utilizando piel de oreja de cerdo,



posteriormente a este período, al área expuesta con la formulación se realizó la técnica *tape stripping*. Todas las muestras fueron cuantificadas mediante espectroscopia de ultravioleta. Finalmente, se desarrolló una forma farmacéutica semisólida como modelo de ensayo para la aplicación tópica.

Resultados/Discusión/Conclusión

Fue posible incorporar 35% de cúrcuma en ambos sistemas (CL y CL-70C). Por otro lado, el pH disminuyó a partir del cuarto mes, lo que puede sugerir una organización en la matriz. Durante los seis meses, todos los sistemas se mantuvieron transparentes y viscosos, los cuales presentaron cruces de malta y estrías indicando la formación de fase lamelar y hexagonal. El DSC de CL-70 presentó dos picos endotérmicos y en el CL-70C se observó dos picos endotérmicos de menor intensidad. De acuerdo con estos resultados, se estima que la cúrcuma puede interactuar con el sistema. Fue posible observar que la forma farmacéutica elaborada favoreció la permeación de *Curcuma longa* hacia la sangre. Con lo expuesto, se sugiere que los CL son sistemas nanoestructurados innovadores para la preparación de diferentes formas farmacéuticas.

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Medicinal plants utilization in the context of chinese medicine

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Introduction

The popular indications of the plants are coherent with the syndromes of the theory of the five phases of traditional Chinese medicine (TCM). The symptoms of these syndromes are related to physiopathological alterations of modern medicine. The purpose of this work is to propose a strategy for the use of medicinal plants based on the theory of the five elements of TCM.

Method

The indications of 60 medicinal plants of the American and European floras were compiled and grouped according to the physiological and physiopathological systems of modern medicine to which they correspond. The components of the ten syndromes in the system of the five phases of TCM were grouped according to their physiopathological origin. The correspondences were analyzed between the systematized indications of the medicinal plants and the physiopathological components of the TCM syndromes.

Results

The analysis showed that the Wood phase is related to sympathetic function, allergic reactivity, and the neuromuscular plate. The Fire phase is related to cardiac and arterial sympathetic function, the small intestine. The Earth phase is linked to the parasympathetic system, metabolic dysfunctions, and hypophysigonadal alterations. The Metal phase also relates to the parasympathetic function, alterations of the respiratory tract, and the skin. The Water phase is related to the immunological response, urinary alterations, and the trophism and functioning of the neuronal tissue.

Discussion and Conclusion

The traditional and popular indications of medicinal plants show a high degree of correspondence with the syndromes of the theory of the five phases of TCM. These coincidences allow developing a coherent system of the use of medicinal plants.



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Evaluación del potencial antioxidante de diferentes hierbas aromáticas y de una bebida tradicional del Ecuador

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Introducción

Ecuador está entre los 17 países más biodiversos del mundo, con más de 16000 especies de plantas (Coba & Tivi, 2010). En este sentido, existen 5172 plantas útiles, de las cuales 3118 especies han sido usadas durante siglos por numerosas comunidades con fines medicinales, y la mayoría de estas plantas medicinales son hierbas, arbustos y árboles (De La Torre, Balslev, Navarrete, & Macía, 2008). Uno de las bebidas más populares de la región sur del Ecuador es la Horchata Lojana, preparada a partir de una infusión de una mezcla de 15 o más hierbas y flores aromáticas. El presente trabajo tuvo como objetivo cuantificar la capacidad antioxidante y el contenido de fenoles totales de extractos obtenidos a partir de los 20 ingredientes que conforman la Horchata Lojana, y la bebida final.

Método

Se trabajó con las especies vegetales: Menta (*Mentha spp.*), Albahaca (*Ocimum basilicum*), Esencia de rosas (*Pelargonium graveolens*), Escancel (*Aerva sanguinolenta* L.), Shullo (*Oenothera rosea*), Pena Pena (*Fuchsia spp.*), Linasa (*Linum usitatissimum*), Hierbaluisa (*Cymbopogon citratus*), Cola de Caballo (*Equisetum giganteum*), Toronjil (*Melissa officinalis* L.), Violeta (*Viola yedoensis*), Llantén (*Plantago spp.*), Pimpinela (*Poterium sanguisorba* L.), Ataco (*Amaranthus spp.*), Manzanilla (*Matricaria chamomilla*), Borraja (*Borago officinalis* L.), Malva Blanca (*Lavatera arborea* L.), Clavel (*Dianthus caryophyllus* L.), Cedrón (*Aloysia triphylla*) y Cucharilla (*Oreocalis spp.*), además con la mezcla de todas los ingredientes combinados en una proporción que se acostumbra a comercializar en los principales mercados de la ciudad de Loja - Ecuador. Para extraer los antioxidantes de las muestras, se empleó el método descrito por Saura-Calixto y Pérez Jiménez (2007). Para la determinación de la capacidad antioxidante se emplearon los métodos de DPPH, ABTS y FRAP, descritos por Thaipong et al. (2006). Los resultados se expresaron en μmol equivalentes a trolox (TE)/g BS. La determinación de fenoles totales se realizó mediante el método Folin- Ciocalteu, que fue descrito por Singleton (1965). Los resultados se expresan en mg equivalente de Ácido Gálico (EAG)/100g BS. Se realizaron análisis estadísticos y comparaciones de medias utilizando el paquete estadístico MINITAB 16.0 software (PA, USA).



Resultados/Discusión/Conclusión

La Horchata Lojana mostró una alta actividad antioxidante (AA) medida por los métodos DPPH, ABTS y FRAP de 676 ± 29 ; 458 ± 7 y 409 ± 7 $\mu\text{mol TE/g BS}$. Los ingredientes que presentaron mayor AA fueron las especies pena pena con valores de 2300 ± 14 ; 2213 ± 20 y 1332 ± 23 $\mu\text{mol TE/g BS}$, respectivamente. Esencia de rosas con 1851 ± 47 ; 1600 ± 70 ; 1076 ± 42 $\mu\text{mol TE/g BS}$. Shullo con 1716 ± 78 ; 1490 ± 30 ; 789 ± 42 $\mu\text{mol TE/g BS}$. Respecto, al contenido de fenoles totales (TPC), la Horchata posee un valor de 5515 ± 218 mg EAG/100g BS. Además, se realizó la preparación de la infusión, encontrándose un valor de 202 ± 6 mg EAG/L, concentración comparable con la encontrada en un vino blanco 282 ± 10.7 mg EAG/L. Por otro lado, los ingredientes que presentaron el mayor TPC son pena pena, esencia de rosas y shullo con valores de 16530 ± 426 ; 12999 ± 543 y 12107 ± 1060 mg EAG/ 100g BS. Se concluye que la bebida tradicional Horchata Lojana posee una alta actividad antioxidante y alto contenido de fenoles totales, de forma tal que puede ser considerada con una bebida funcional.

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***Arecaceae* del sur del Ecuador como fuente de tocoferoles y tocotrienoles**

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Introducción

Las palmas son uno de los elementos más conspicuos en muchos tipos de vegetación tropical y subtropical (Balick 1983, Henderson et al. 1995). La familia de *Arecaceae* (palmeras), se pueden desarrollar en una gran variedad de climas. Existen aproximadamente 33 géneros conocidos, actualmente con alrededor de 127 especies y 15 especies endémicas de *Arecaceae* en el Ecuador (Jørgensen, León, 1999). En nuestro país fueron usadas por siglos por los indígenas, ayudándoles a suplir sus necesidades diarias como: alimentación, vivienda, herramientas, bebidas, medicina, etc. En las provincias de Loja y Zamora Chinchipe ubicadas al sur del Ecuador habitan muchas especies de palmeras de las cuales no existe evidencia de estudios fitoquímicos previos, siendo éstas una fuente natural de vitamina E, la cual tiene una función antioxidante (Dewick P. 2001), contribuyendo a prevenir el envejecimiento de células, tejidos y algunas formas de cáncer (Watson, Preedy, 2009). El objetivo de este proyecto realizar la identificación de la composición química de los aceites vegetales de las diferentes especies de *Arecaceae* mediante Cromatografía de Gases acoplado a Espectrometría de Masas.

Método

Se recolectaron 4 especies de *Arecaceae*. *Mauritia flexuosa*, *Wettinia maynensis*, *Prestoea acuminata* y *Veitchia merrilli*

La extracción de los aceites vegetales fue realizada mediante equipo Soxhlet utilizando los frutos de las palmeras. Por cada 40 g de muestra, se añadió 200 ml de hexano. Se dejó destilar por 2 h a 70 °C. Luego se concentró en un rotavapor a 25 °C y los aceites se almacenaron en refrigeración a una temperatura de -4 °C (Amaral, 2005).

Se determinaron propiedades como la densidad, apariencia e índice de refracción por normas INEN.

La identificación de tocoferoles y tocotrienoles se realizó mediante los procesos de saponificación, derivatización e inyección de la muestra empleando la técnica analítica de Cromatografía de Gases acoplada a Espectrometría de Masas (GC/MS), en donde se utilizaron los espectro de masas de cada especie, basándonos tanto en el ion de referencia, como en la característica específica para la identificación. Los espectros de masas se compararon con los espectros de estándares y de estudios previos.

Resultados/Discusión/Conclusión

Mediante GC/MS se identificó en la especie *Veitchia merrilli* el α -tocoferol, al que le corresponde un tiempo de retención de 12,19 min. En el espectro de masa se observó el ión molecular m/z 502, con fragmentos m/z 237 y 277. En la especie *Mauritia flexuosa* se identificó el β o γ -tocoferol y el α -tocoferol, a éstos les corresponde un tiempo de retención de 12,12 y 10,04 min, respectivamente. En el espectro de masa se observó el ión molecular m/z 488, con fragmentos m/z 223, 263 que corresponde al β o γ -tocoferol en esta muestra y también se observó el ión molecular m/z 502, con fragmentos m/z 237, 277.

Los resultados obtenidos en esta investigación fueron comparados con (Mirmira, Edward, 1972), donde se estudia la presencia de tocoles en aceites vegetales y se puede evidenciar muy claramente que obtienen los mismos iones moleculares que en el presente estudio.

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Efectividad de la tintura de propóleo al 4% en afecciones de la faringe y amígdalas

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Introducción

Desde de la antigüedad se han mencionado las propiedades del propóleo, los egipcios lo utilizaron como uno de los ingredientes para conservar las viseras de los faraones y en la curación de las heridas u otras lesiones de guerra conjuntamente con la miel y como antibiótico natural.

El trabajo describe los resultados novedosos del empleo de la tintura de propóleos al 4 % en afecciones de la faringe y amígdalas durante el año 2018 en pacientes tratados en el Hospital Clínico-Quirúrgico-Docente “Octavio de la Concepción y de la Pedraja” de la Ciudad de Camagüey.

Materiales y Métodos

El propóleos empleado corresponde a la variedad marrón oscuro, suministrado por la APISUM de Camagüey. Para la maceración al 4% se emplea una proporción equivalente a 4 peso-volumen de propóleo y alcohol de 95 grados en percoladores durante un período de 7 días, agitando con frecuencia y filtrado con un filtro de poro fino. La tintura obtenida es embasada en frasco ámbar protegido de la luz y se almacena a temperatura ambiente en lugar fresco por períodos no superior a un año. Se administran 20 gotas diluida en 120 ml de agua tibia, en forma de gargarismos. Las afecciones tratadas fundamentalmente han sido faringitis aguda, atrófica, alérgica y granulomatosa, así como, amigdalitis eritematosa, pultácea y virales.

Resultados

Durante el año 2018 se atendieron en la consulta de otorrino un total de 529 pacientes a con prescripción del empleo de la tintura del propóleo para las afecciones descritas. Se pudo constatar una apreciable mejoría entre la 48 a 72 horas y una recuperación total al séptimo día, sin que se produjeran reacciones adversas. El trabajo incluye el correspondiente tratamiento estadístico.



Se concluye que resulta efectivo el empleo de este apifármaco para el esquema de tratamiento descrito.

Activity against the fungus *Colletotrichum lindemuthianum* by synthetic compounds structurally related with bean phytoalexins

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Introduction

Anthrachnose is a worldwide disease of legumes caused by the fungus *Colletotrichum lindemuthianum*. The disease is most common and severe on common bean (*P. vulgaris*) but may also affect lima bean (*P. lunatus* L.), scarlet runner bean (*P. multiflorus* Willd.), mung bean (*P. aureus* Roxb.), cowpea (*Vigna sinensis* Savi.), and broad bean (*Vicia faba* L.). The causal organism affects the quality of pods and grain, and consequently reduce the market value of crops. Overall, anthracnose can produce yield losses ranging between 38% and 95% depending on bean species and susceptibility of cultivar. Actually, one of the alternatives that are being more intensely explored is based in the natural protecting mechanisms of plants through the production of phytoalexins. Phytoalexins, secondary metabolites synthesized *de novo* by plants after a pathogen infection have shown a broad spectrum against microbes. However, have been demonstrated that many of these substances can be rapidly metabolized by many microorganisms, which make them practically harmless products. For this reason, a potential alternative of phytoalexins consists in design new protectants based on their structural nucleus. From the structure of phytoalexins it is possible to develop synthetic analogues with improved properties, such as antifungal activity. For the above, in the present work we report the antifungal activity of some structurally modified bean phytoalexins. Specifically, isoflavonoids phytoalexins were selected as structural template to design the analogues.

Method

A series of compounds such as 3-formylchromones, benzo [d] thiazolyl acetonitriles, aryl-2-propen-1-ones, thiochromanones and thiochromanone acylhydrazones, 3-formylchromones and their imines were prepared by different reactions (hydrazone formation, Knoevenagel condensation, Michael reaction, and aldolic condensation). Each compound synthesized was purified by chromatographic techniques (TLC and CC) and identified by spectroscopic techniques (¹H y ¹³C NMR 1D). The antifungal activity was evaluated against *C. lindemuthianum* through the poisoned food technique. The analysis was determined as growth inhibition mycelial and germination of spores.



Results/Discussion/Conclusion

The results of the biological activity showed that some compounds have high fungistatic activity, among which are some thiochromanones, 3-formylchromonas and benzothiazolil acetonitriles. For this, it can be established that these substances can be used for the development of new antifungal compounds for the control of anthracnose in beans.

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Chemical composition of essential fruit oil of *Pittosporum undulatum* from Colombia and evaluation of its repellent activity against *Pullex irritans*

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Introduction

Pittosporum undulatum (Pittosporaceae), commonly known as the "galán de noche" or laurel huesito, is an ornamental shrub native to Australia and grown in temperate climates around the world; Its fruits are rounded or slightly flattened capsules of green, yellow or orange color as they mature. Chemical studies of its essential oils have reported the presence of calamenene, farnesol and spathulenol as major components present in the leaves (Medeiros, 2002). Bearing in mind that the repellent activity of essential oils has been demonstrated in innumerable studies, as well as the importance of the synergistic effects that their components have (Nerio et al., 2010); the present work is oriented to the study of the chemical composition of the essential oil extracted from the fruits of *Pittosporum undulatum* and the evaluation of its repellent activity against *pullex irritans*, an ectoparasite that can cause pathologies because it is a hematophagous vector.

Method

The plant material was collected in the city of Bogotá (Geographical Coordinates 4 ° 42'37.7 "N 74 ° 07'29.5" W), during the month of August 2018. A control sample was sent to the National Herbarium of Colombia for its determination taxonomic which was classified as *Pittosporum undulatum*, under COL218 collection code. The essential oil was obtained from 1000 g of fresh fruits by steam distillation with water for 4 hours, obtaining 2.5 ml of oil with a yield of 0.25%. The chemical composition of the oil was determined by gas chromatography coupled to mass spectrometry (GC-MS) and the identification was made through the calculation of the retention indexes (IR) and comparison with the mass spectra stored in the library NIST08. The repellent activity of the essential oils was evaluated by the area of preference method described by Tapondjou et al., 2005, using sheets of 7 cm filter paper Whatman cut in half (19.25 cm²). The test solutions were prepared in acetone obtaining the doses of 0.05, 0.10, 0.20 and 0.40 μ l/cm². The other half of the filter paper was treated with acetone and used as a control. In each of the reconstituted discs 10 insects were

released; the treatments were replicated 5 times and the numbers of insects present in the control (Nc) and treated (Nt) areas of the discs after 2 hours were recorded.

Results/Discussion/Conclusion

In the essential oil 23 compounds were identified (63.59%), of which 15 correspond to oxygenated monoterpenes (22.71%), 3 sesquiterpenes (4.81%), 3 ketones (1.23%), 1 aldehyde (0.39%) and 1 monoterpene (34.45%), this being the majority component identified as D-limonene. The repellency bioassay allowed establishing that the dose of 0.10 $\mu\text{g}/\text{cm}^2$ exhibited 90% repellency of the essential oil against *pullex irritans*; which suggests the essential oil of *Pittosporum undulatum* fruits as a possible promising source for the treatment by infestation of this ectoparasite.

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Craft beer aging fingerprint by using e-tongue

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Introduction

With the increasing consume and export of beer, shelf-life problems have become a very important issue for most breweries. Beer, and above all craft beer, is an unstable beverage and during its shelf-life a series of chemical, physical and sensorial transformations occur. Beer aging is a complex phenomenon characterized by a number of complex reactions involving proteins, carbohydrates, polyphenols, metal ions, oxygen, thiols, carbonyls. The extent of these reactions depends on the production process, storage conditions and interaction of pathways. Even if compounds with antioxidant properties such as reducing sugars, Maillard reaction products, vitamins and above all polyphenolic compounds, important antioxidants with mechanisms involving both free radical scavenging and metal chelation, are present in beer, all these compound have a negligible effect on the oxidative stability of beer during storage and, then quality of beer decreases and production of undesirable flavors occur. Furthermore, several studies show that self-oxidation, including the decomposition of iso-a-acids, plays an important role in deterioration of the flavour and aromatic qualities of beer during aging. In particular, the loss of the bitterness of beer may be due to the degradation of iso-a-acids, in particular to the instability of trans-iso-acids.

The aim of this preliminary study was to explore the potential of using potentiometric electronic tongue as a tool for evaluating craft beer shelf-life based on the analysis of taste profile modifications.

Method

The e-tongue analysis were performed with the α -Astree electronic tongue (Alpha MOS company), that consisted of seven different liquid cross-selective potentiometric sensors (JB, BA, BB, HA, ZZ, CA and GA) (Alpha M.O.S.), an Ag/AgCl reference electrode (Metrohm, Ltd). The sensors used are chemically sensitive field-effect transistors (chemFET). Also, the chemical-physical parameters (color, pH, bitterness) were determinated. Two different types of craft beers have been studied: blonde red. All the obtained data were analyzed individually and then a data fusion was carried out.

Conclusion



An E-tongue system based on potentiometric chemical sensors was assessed as a rapid tool for studying blond and red craft beer shelf-life. Statistical technique SQC was run on the data matrix of e-tongue sensors response and the results showed that in a few months of storage blonde beers present substantial changes in the taste profile while changes red beers occur in a longer time, after the expiry date shown in label.

For the validation of the developed e-tongue test, specific chemical-physical analyzes were conducted. The changes detected over time in the chemical-physical parameters of the two types of craft beers confirm the potential use of the electronic tongue combined with a robust statistical elaborations as a tool for monitoring craft beer shelf life.

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Effect of different sources of fertilizers on selected health beneficial bioactive compounds of *Topepo pepper*.

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Introduction

Among vegetables, the genus *Capsicum annum* (pepper) comprises a large number of species cultivated worldwide because of its fruits are an excellent source of health-bioactive compounds, such as ascorbic acid, carotenoids, tocopherols phenolic compounds and flavonoids. Pepper fruits are generally consumed fresh and/or cooked and their extracts are also used in pharmaceutical fields. Among capsicum genus, Topepo red sweet pepper, is a cash crop ranking in the first positions of the world agriculture for its high nutritional and economic values. Topepo red sweet pepper fruits are a rich source of vitamin C, polyphenols, chlorophylls, carotenoids, sugars as well antioxidant bioactive compounds whose amounts and types can highly modulated also by the type of fertilization used. Numerous researches have been mainly focused on discriminating the effects of organic and inorganic fertilizers on productive characteristics of crops, but few studies widely evaluated the effects of the different types of fertilizations on the content of bioactive nutrients or of compounds with antioxidant properties. For the above statements our aims were: 1) to discriminate the effects of organic from inorganic fertilizers on Topepo pepper fruits secondary metabolism and antioxidative system; 2) to compare the effects of two composts differing in composition with horse dung, an organic fertilizer widely used in agriculture for the cultivation of pepper.

Method

The effects of different organic fertilizers: the commercial horse dung (HD) and compost obtained from wastes of traditional olive oil production systems (CO) and from residues of fourth range vegetables industry (CV) were evaluated on the quality of topepo pepper, analyzing phytochemicals, aromatic fraction and antioxidant capacity in respect to the un-fertilizer pepper (Control). The experiment was arranged in a randomized complete block design and replicated three times.

Results/Discussion/Conclusion

The composts produced with the different wastes had different chemical composition, CO had the highest amount of carbon and nitrogen while the lowest cation exchange capacity (CEC). Conversely CV was richest in nutrients and phenols and had the highest CEC, identifying CV as compost with the best quality. The compost properties affected differently the synthesis of bioactive compounds in topepo pepper. At the end of 15 grown weeks, it was observed that the application of CV enhanced the production of total phenols, flavonoids, ascorbic acid, vitamin, carotenoids, anthocyanins, carbohydrates as well as volatile fraction compared to HD, CO and CTR. The DPPH, ABTS, FRAP and Ferrozine activities were also significantly the highest in topepo pepper grown with CV. The results indicated that the use of compost from vegetable residues can enhance the production of secondary metabolites and improve the antioxidant capacity of Topepo pepper increasing in a sustainable way its nutraceutical and economic value.

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Craft beer's volatilome fingerprinting by headspace solid-phase microextraction and gas chromatography-quadrupole time-of-flight mass spectrometry (HS- SPME- GC- QTOF/MS)

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Introduction

Beer is a complex mixture of constituents, brewed from raw materials including mainly malt, water, yeast, hops and contains a broad range of different chemical components that may react and interact at all stages of the brewing process. Aroma substances are very important in beer as they make a major contribution to quality of the final product and has great importance in consumers' preferences. Beer aroma profile is characterized by a complex mixture of many components at very low concentration which are responsible of its distinctive personality.

The aim of this work was the development of a new method to trace the craft beer aromatic fingerprinting produced in Calabria (south Italy) in comparison with commercial beer. Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-quadrupole time-of-flight mass spectrometry (GC-QTOF MS) for the first time, to our knowledge, has been used in the aroma beers tracing. Compared with the static HS extraction and the different SPME fiber coatings, HS-SPME with divinylbenzene/carboxen/polydimethylsiloxane fiber was preferred to extract the target analytes including aldehyde, alcohol, ester and other classes of compounds previously identified in the aroma of beer. In the identification, a multi-dimensional qualitative analysis approach containing library searching, retention index comparison, accurate mass measurement and area normalization was utilized.

Method

Then volatile compounds extraction was carried out by a 50/30 μm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber for autosampler. A GC (Agilent 7890A) coupled into a QTOF accurate mass selective detector (Agilent, 7200) was used to analyze the sample headspace components.



MassHunter software B.08 was used for the control of equipment and data acquisition and The Unknown analysis software was used for tentative compound identification by comparing mass spectra with NIST11 library data.

Conclusion

The analysis of scientific literature shows how the study of the aroma of beer, due to the many variables that contribute to its formation, is complex. Based on the low-resolution mass spectra solely, it was difficult to distinguish the volatile compounds correctly. To resolving these problems, GC-QTOF MS was employed to make a structural confirmation via the accurate mass measurement. Through the Unknown Analysis software based on the use of NIST database search, 50 compounds were identified. The study conducted allowed us to identify the *aromatic fingerprinting* of both commercial and craft-produced beers, highlighting both the discriminatory potential of the method developed and the richness and high quality of craft beers produced in Calabria.

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Aromatic fingerprint of DOC red wine from autochthonous *Gaglioppo* cultivar by a metabolomics approach using a headspace solid-phase microextraction and gas chromatography-quadrupole time-of-flight mass spectrometry

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Introduction

The final sensorial quality of a wine, and in particular the volatile fraction that characterizes its aroma, depend on different factors both endogenous and exogenous. Among the endogenous factors the main role is played by genetics, and therefore by the grape cultivar, while among those exogenous the environment, the cultivation practices and, above all, the wine production process and aging. All aroma compounds may play a role in the characterization of the specific flavor pattern of each wine. Wine contains a very large number of aroma-active compounds like terpenes, alcohols, esters, methoxypyrazines, aldehydes, which impart distinct aromas such as floral, fruity, woody or pepper. The unique and non-linear interaction between these chemical compounds determines the final aroma of a wine. To our knowledge there are no specific studies on volatility of DOC wines obtained in purity from *Gaglioppo* cultivar grapes according to the traditional process codified in the disciplinary. Therefore, this study, with the aim of characterizing the volatility fingerprinting of DOC wines obtained with *Gaglioppo* grapes, reports the development of a non-targeted volatile metabolomic approach based on GC-QTOF/MS coupled with solid phase microextraction.

Method

Volatile compounds extraction was carried out by a 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber for autosampler. A GC (Agilent 7890A) coupled into a QTOF accurate mass selective detector (Agilent, 7200) was used to analyze the sample headspace components. MassHunter software B.08 was used for the control of equipment and data acquisition and the unknown analysis software was used for compound identification by comparing mass spectra with in the Mass Spectral Library of National Institute of Standards and Technology (NIST11 library data).

Conclusion

The aromatic profile of analyzed wines were extremely complex. More than 100 aroma compounds have been traced (most of them in a very low concentration) and through the Unknown Analysis Software, based on the use of NIST database, search 35 compounds were identified.

The aroma bouquet of *Gaglioppo* wine is a complex interaction between numerous volatile chemical compounds and these compounds interact with each other in various ways to achieve the final aroma. Volatile esters constitute one of the most important classes of aroma compounds and are largely responsible for its fruity aroma.

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Feasibility study on the potential of e-tongue in craft beer discrimination and nutraceutical description

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Introduction

Beer is the world's most widely consumed and probably oldest alcoholic beverages. It is the third most popular drink overall, after water and tea. Beer is obtained by fermentation of starch-rich wort from cereal grain, mainly malted barley, but also wheat, maize, and rice. Most beer is flavoured with hops, which add bitterness and act as a natural preservative. Other flavourings such as fruits or herbs may be included especially in craft beers. Beer alcoholic content is between 3 and 8% and can be considered a good source of polyphenols derived both from malt and hop. There is a wide variety of beers produced all over the world, however, there is an effort to differentiate and classify them taking into account both chemical composition and production techniques and mainly between commercial and craft beers.

In this work is presented a feasibility study on the potential of a potentiometric electronic tongue as a tool for fast discrimination of different styles of commercial and craft beers and of their nutraceutical characteristics.

Method

The E-tongue analysis were performed with the α -Astree electronic tongue (Alpha MOS company), that consisted of seven different liquid cross-selective potentiometric sensors (JB, BA, BB, HA, ZZ, CA and GA) (Alpha M.O.S.), an Ag/AgCl reference electrode (Metrohm, Ltd). The sensors used are chemically sensitive field-effect transistors (chemFET). Also, the physicochemical parameters color, pH, bitterness were measured. Measurements were carried out on three styles (blonde, red and weizen) of commercial and craft beer. All the obtained data were analyzed individually and then a data fusion was carried out.

Conclusion

There are some differential aspects in the composition and organoleptic characteristics among the different styles. Among the substances that contribute to beer characteristics, especially to color, there are a large number of active compounds which include polyphenolic compounds. Normally, most polyphenolic compounds, mainly phenolic acids, flavan-3-ol derivatives and flavone glycosides, come from malt and a residual part from hops. There is a relationship between the content of polyphenols and a relationship has



also been established between free phenols and phenolic acids content and the antioxidant activity. Due to its antioxidant capacity and low alcoholic content, beer is able to improve plasma antioxidant activity and reduce the risk of cardiovascular diseases without the negative effects of high doses of alcohol.

PCA, an unsupervised statistical technique, was run on the data matrix of physicochemical and e-tongue sensors response to discriminate between craft and commercial beer and nutraceutical characteristics. The samples, craft and commercial beer, are clearly separated. The first and the second principal components account for 38,1% and 29,6% of the total variance, respectively. The main contribution to the first component is due to the pH and bitterness. Separation along the second principal component is mainly caused by the colour and two e-tongue sensor response (CA, HA). This work confirm the ability of biomimetic systems combined by robust statistical elaborations for discriminant model able to classify beer by styles and nutraceutical quality.

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Validación de bioactividad del follaje de *Bursera simaruba* sarget (almácigo)

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Abstract

La especie *Bursera simaruba* es un árbol medicinal muy arraigado en la cultura popular cubana por su potencial curativo, tanto desde el punto de vista de la medicina tradicional como de la religión yoruba el almácigo es reconocido como árbol sanador y purificador con el que no se hace nada malo. El objetivo del trabajo es estudiar la actividad antiviral y antioxidante del follaje de *Bursera simaruba*. A partir del material seco y molido se obtuvieron cuatro crudos para la evaluación: de n-hexano, etanol, acetato de etilo y butanólico. Los ensayos de actividad biocida se realizaron “*in vitro*” sobre el virus HSV-2 (aislamiento clínico). Se evaluó el parámetro de viabilidad celular frente a los crudos mediante el cálculo de los valores de concentración citotóxica media (CC₅₀) y se determinó la actividad antiviral a partir del índice selectivo (IS). Se determinó el contenido de fenoles totales mediante método espectrofotométrico que utiliza ácido gálico como estándar (0 a 500 mg/L) a partir de los resultados se evaluó la capacidad antioxidante, expresada en equivalentes de Trolox por masa de material vegetal seco, utilizando 2,2-difenil-1-picrilhidrazilo (DPPH). Se demostró la actividad antiviral de la fracción más polar (crudo butanólico) al inhibir la replicación del virus del herpes simple tipo 2 “*in vitro*” mientras que los crudos etanólico y de acetato de etilo mostraron los valores más elevados tanto en el contenido de fenoles totales como en la evaluación de capacidad antioxidante.



Development and registration of a shampoo, a capillary lotion and a talk for feet with *Melaleuca quinquenervia* oil

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Modalidad: Cartel.

Temática: Investigación clínica, desarrollo, comercialización y marketing de productos naturales

Abstract

Cosmetic products, enriched with natural substances, constitute an excellent possibility to preserve, improve and demonstrate human beauty and health. The benefits of Melaleuca Oil are used in several pharmaceutical and cosmetic products for its many benefits, among which are its repellent, acaricidal, fungicidal and bactericidal action, properties that are important for developing and registering various cosmetic products such as talc fungicide for Feet, germicidal shampoo and effective hair lotion against nits and lice as the main objective of the research and development direction of the company. Three products that meet the quality and toxicology requirements established in the regulations for these products were obtained, issuing the Mela max Germicidal Shampoo Registration Certificates, the Mela plus Hair Lotion and Talcomel deodorizing and drying Talc, by the National Institute of Food Hygiene (INHA). Surveys were conducted on people of both sexes and different ages to evaluate each product, having 100% general acceptance. The deodorizing and drying power of Talc for the Feet, the ability of the shampoo to achieve excellent cleaning and foam was verified, the Hair Lotion was 100% effective, no formulating caused adverse reactions, being highly demanded for being a daily need in school sectors, grandparents' house, psychiatric hospitals, prisons, among others.





Aplicaciones de los mucílagos en el sector agroalimentario

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Introducción

Los mucílagos, hidrocoloides de naturaleza viscosa, presentan propiedades como agentes suspensores, espesantes, aglutinantes y clarificantes que resultan de interés para investigadores y productores de los sectores cosmético, farmacéutico y agro-alimentario. Teniendo en cuenta estas propiedades, en el presente trabajo se analizan algunas de las aplicaciones de los mucílagos obtenidos a partir de fuentes vegetales en la industria agro-alimentaria.

Método

En Ecuador, se han empleado los mucílagos obtenidos a partir del cadillo (*Triumfetta lappula* L.), balso blanco (*Heliocarpus americanus* L.), balso rojo (*Ochroma pyramidale*), malva silvestre (*Malva peruviana* L.), malva morada (*Lavatera arbórea* L.), abrojo (*Bittneria ovata* Lam.), cucarda (*Hibiscus syriacus* L.), falso Joaquín (*Hibiscus rosa sinensis*); nieve (*Calystegia soldanella*), uyanguilla (*Basella alba*), moquillo (*Saurauia bullosa* Wawra), yausa (*Abutilon insigne* Planch.) y yausabara (*Pavonia sepium* A. St-Hil) en la clarificación de jugo de caña.

Dos de los agentes naturales más estudiados por su capacidad de coagulación en los procesos de tratamiento de agua potable son la *Moringa oleífera* y diferentes tipos de cactus, que constituyen una alternativa ambientalmente sostenible. También se han utilizado mucílagos, como el de la penca de tuna, en el proceso de coagulación-floculación de aguas turbias.

Se realizó la optimización de la extracción del mucílago del nopal (*Opuntia ficus-indica* var. Forrajera) para su aplicación como recubrimiento comestible en la conservación de jícama cortada. El procedimiento que incluyó el escaldado de la penca pelada y molida fue el que dio un producto con mejores características para su uso como recubrimiento. Además, se evaluó el efecto de la adición de ácido oleico y ácido esteárico en las propiedades de películas a base de este mucílago. Ningún recubrimiento redujo la pérdida de masa del producto durante su almacenamiento a 4 °C y 85 % de humedad relativa, aunque disminuyó su pérdida de firmeza.



Varios trabajos han reportado la aplicación de Aloe vera como recubrimiento comestible en frutas y hortalizas como uvas de mesa, cerezas dulces, nectarina Arctic Snow, tomate. También se ha empelado coberturas de alginato de sodio con Aloe vera en la calidad de zanahoria mínimamente procesada y tomates.

Resultados/Discusión/Conclusiones

Los mucílagos obtenidos a partir de fuentes vegetales presentan propiedades de interés para los sectores cosmético, farmacéutico y agro-alimentario. Entre las principales usos se encuentran su utilización como agentes espesantes y clarificantes, además de aplicarse como coberturas comestibles en la conservación de productos hortofrutícolas enteros y mínimamente procesados.

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Photoprotection and moisturizing properties of cosmetic products based on extracts from the Colombian Caribbean algae *Hypnea musciformis*

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Introduction

During the last years, the cosmetic industry has shown a steady growth. In Colombia, the Chamber of Commerce registered a monetary movement of 3280 millions of dollars associated to this industry. However, the development of new products is limited to the ability to find bioactive compounds with significant properties. Often, these compounds are secondary metabolites of plants and algae. Amongst the numerous benefits associated to the secondary metabolites of Macroalgae, there are the UV protection, and prevention of uneven texture and looseness of the skin. The red algae *Hypnea musciformis* is abundant in Colombia and our results show a significant content of antioxidant and moisturizing compounds on its extracts. Therefore, we developed cosmetic products based on the secondary metabolites obtained from this algae. We hope these products create more economic opportunities in benefit of the coastland peoples in Colombia.

Methods

New products: We used super critic fluid extraction at 1 atm and ethanol content of 95% in order to obtain the two bioactive compounds previously identified as present in *H. musciformis*. Then, we combined these compound with the basic components in compliance with the European regulation N. 1223, producing a moisturizing cream, sunscreen, and a facial mask. We added UV filters, *H. musciformis* extracts, vitamin A, B, y D, collagen and aloe.

Testing: We measured the photoprotection and moisturizing properties of our cosmetic products.

Physicochemical properties: we measured density, viscosity and pH of our products.

Microbiology tests: We evaluated the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and coliform bacteria.

Organoleptic tests: We measured product approval according to colour, smell and texture.

Results/Discussion /Conclusion

We obtained extracts with high antioxidant activity (550 μmol equiv Trolox/L) and with a content of carragean of 45% p/p, which correspond to the gelling agents. Our measurements showed high absorbance at the wavelengths 190 nm, 290 nm and 365 nm. Therefore, we conclude our products can protect the skin from damage caused by UVC. UVA and UVB. The relative densities of the facial mask, moisturizing cream and sunscreen were 1.0062, 0.9337 and 0.9802 respectively. The pH measured for our products was 6.90, 6.86 and 6.80 respectively, all of them are close to neutral values, which means none of our products will cause irritation in the skin. Regarding viscosity, both our moisturizing cream and sunscreen showed a behavior of a pseudoplastic fluid, while the face mask had a viscosity value of 0.7461 cP. These characteristics enable a pleasant texture and application of the products. The skin irritation test in vivo, showed 100% of negative results confirming that our products do not cause any harm to the skin. The application of our products caused a significant increase of moisture in the skin. Finally, our three products obtained significant positive scores in all the test of colour, smell and texture

H. musciformis extracts can be regarded as effective ingredients to be used in cosmetic products for sun protection and moisturizing. The mixture of vitamins, aloe and collagen, used in combination with algae extracts has a synergic effect improving the properties of core compounds of cosmetic products. The good yielding of sun filters can prevent the DNA damage induced by UV radiation. In conclusion, our products showed high standards performance and highlight the benefits of red algae extracts in protecting the skin due to their antiaging properties.

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Acute and chronic activity of a nonpolar extract of *Salvia amarissima* Ort. in type 2 diabetes mellitus mice.

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Introduction

Diabetes mellitus is one of the main health problems in Mexico, it causes high cases of mortality, incapacities and elevated cost to the Mexican health system (1.1% of PIB of the country at the year 2013).^{1,2,3} Due to this causes, is important to find new treatment alternatives o improve the quality of life of the people and reduce the cost of the treatments. In this case the plant specie *Salvia amarissima* of wide distribution in Mexico⁴ could be as source of new products for the treatment and control of diabetes mellitus, this specie is used in some Mexican communities with ethnomedical use as antidiabetic.⁵

Methods

Animals: Male Balb-c mice were used. **Extracts:** Fresh aerial parts of *S. amarissima* were extracted with petroleum ether. **Induction of experimental diabetes:** Induction was realized by an intraperitoneal administration of streptozotocin (100mg/kg) and nicotinamide (240mg/kg), animals with glucose of 280-370 mg/dL were used for the assays. **Treatments:** The extract was administrated for the acute assay at doses of 50, 100 and 300mg/kg and for the chronic assay at dose of 100mg/kg, both of them were administrated intragastric, evaluation time was at time 0,1,3,5 and 7 hours after the administration, for th acute assay, for chronic was evaluated every 7 days for eight weeks. The blood glucose was measured with and Accu-check performa glucometer, by a puncture in the tail of the animals.

Results/Discussion/Conclusions.

In the acute assays the doses with mayor effect was at 50mg/kg in diabetic mice, this dose was selected for the chronic assay. The acute assay indicates that there are a reduction in the blood glucose levels from the first hour (-55mg/dL) with the higher activity between 3 and 5 hours (-80mg/dL) and maintained to the

7th hour (-45mg/dL) in the case of the other doses the maximum peak was at only one hour and the maximum reduction of -50mg/dL for the dose of 100mg/dL and -27mg/dL for the dose of 300mg/kg. In the chronic assay animals with blood glucose of 370mg/dL have a reduction of the blood glucose from the first week having statistical significance at the 4th week of treatment (229mg/dL) and maintained under the initial levels until the end of the assay (285mg/dL).

The treatment with the products obtained from the non-polar extract of fresh aerial parts might be useful as an alternative and effective treatment for diabetes mellitus

Indicando que el tratamiento con los productos no polares de la *Salvia amarissima* Ort. pueden servir como un tratamiento antidiabético alternativo eficaz y seguro.

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Extended evaluation of the acetic acid-induced writhing test

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Introduction

The determination of possible analgesic agents in extracts from medicinal plants depends on the methods available in animals (Siegmund et al., 1957). The tests currently used to detect analgesic agents employ nociceptive stimuli that are known to cause pain and respond to the use of analgesics of known activity in humans. These painful stimuli in turn produce a response in animals, and this response is what indicates the magnitude of pain or analgesia. The acid-induced writhing test is used to evaluate visceral pain, this test is non-specific to detect analgesic agents (Pircio et al., 1975), which allows us to identify both narcotic and non-narcotic analgesics. However, it lacks sensitivity to determine mild or moderate analgesic activity, this analgesic magnitude is frequent in phytopharmaceuticals. In this work, we propose an extended evaluation of this test that allows us to detect the mild or moderate analgesic activity of phytopharmaceuticals.

Method

The acid-induced writhing test is traditionally performed with the administration of acetic acid beginning of the test at concentrations that vary in a range of 0.6 to 1.5% (i.p.) in a volume of 10 ml/kg at the beginning of the test. Typically it is evaluated by quantifying the number of writhing during 20 minutes of the test, an writhing involve the contraction of the abdominal wall, pelvic rotation, followed by the extension of the hind limbs (Fontenele et al., 1996; Rejón-Orantes et al., 2013).

In this work we propose the extended evaluation of this test with the following scale: 0. An animal with motor activity (horizontal and vertical exploration); 1. An animal immobile for more than 20 seconds; 2. Abdominal contraction without pelvic rotation; 3. Abdominal contraction, pelvic rotation (trunk rotation), without extension of the hind limbs; 4. Abdominal contraction, pelvic rotation (trunk rotation) and only one extension of the hind limb; 5. Abdominal contraction, pelvic rotation (trunk rotation) with extension the hind limbs. This scale is quantified minute by minute during the 20 minutes of the duration of the test. Finally, the total score reached during the 20 minutes is quantified.

Results/Discussion/Conclusion



The typical evaluation of this test limits the sensitivity to detect phytopharmaceuticals with discrete analgesic activity. Our results suggest that with the proposed evaluation scale, levels of analgesia that would otherwise go unnoticed can be detected. The extended evaluation of this test allows obtaining more detailed information on the behavior of the rodent during the painful stimulus. As confirmed by the use of different doses of dipyron and morphine, the dose / response curve could be established, increasing the sensitivity of the test. The application of the extended evaluation scale will allow the identification of phytopharmaceuticals with slight analgesic activity.

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Bio-guided fractionation of an extract of *Cucurbita ficifolia* bouche, and effect of some fractions on insulin secretion and GLUT-4 expression

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Introduction

Diabetes Mellitus comprises a group of metabolic diseases, characterized by hyperglycemia as a result from defects in insulin secretion by pancreatic cells or action of it in its target tissues. This known as insulin resistance and its present in tissue sensitive such as muscle and adipose tissue. Both tissues play a central role in the regulation of glucose metabolism¹. Chronical hyperglycemia is highly associated with damage and dysfunction of tissues and organs including kidneys, nerves, eyes, heart and vascular system. Although there are different treatments for a multifactorial and complex disease such as Diabetes, the population continues to resort to traditional medicine, which is why it is important to carry out phytochemical studies of the species that are used. *Cucurbita ficifolia* is a specie commonly used to treat diabetes, in previous studies it has been demonstrated to have a wide range of effects, such as hypoglycemic, antioxidant and anti-inflammatory effects. The aim of this work was to carry out a bio-effect directed fractionation of an extract of *Cucurbita ficifolia*, in order to obtain fewer complex fractions and possibly isolate compounds that are responsible of the antidiabetic effects.

Method

Three different extracts of *Cucurbita ficifolia* were elaborated; ethyl acetate extract, acetone and aqueous extract. Those extract were evaluated in an acute test and oral glucose tolerance test, in order to choose the extract with greater activity and make subsequent fractionation. Primary fractions were tested in the oral glucose tolerance test, submitted to further fractionation, and both the fractions and the sub-fractions obtained from this process were evaluated. Insulin concentration was quantified in pancreatic cells by ELISA and GLUT4 mRNA expression levels were determinate in myocytes by qRT-PCR.



Results/Discussion/Conclusion

The aqueous extract showed a hypoglycemic effect in the acute *In vivo* test. However, the fraction resulting from a split in ethyl acetate of the same extract, was found to have anti-hyperglycemic effect in glucose tolerance test. Likewise, its derived primary fraction F25-27 had effect, indicating this fraction might have compounds responsible of antidiabetic effects. On the other hand, the other ethyl acetate derived primary fractions of low to showed an increase in insulin secretion, while only two of them increased the expression of GLUT4. These results suggest that the anti-hyperglycemic and hypoglycemic effects might be given through different pancreatic or extrapancreatic mechanisms of action. These mechanisms involving different tissues or cells, reflecting the efficacy of this vegetal species in the treatment of diabetes.

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Evaluación de la acción antibacterial de un jabón líquido y gel hidroalcohólico usando como activos aceites esenciales del genero citrus frente a cepas implicadas en acné

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Introducción

El acné, aunque tiene un componente microbiano, fundamentalmente es un proceso inflamatorio. Se caracteriza por presentar comedones, pápulas y pústulas, aunque eventualmente puede haber abscesos, quistes y cicatrices. En la patogénesis del acné confluyen fundamentalmente cuatro procesos: incremento en la producción de sebo, hiperqueratinización perifolicular y obstrucción folicular, colonización por *P. acnes* y otras bacterias oportunistas (*S. aureus*, *S. epidermidis*) y liberación de enzimas.¹ Esto produce un cambio en el patrón de la queratina pilosebácea, que se hace más densa, bloquea la salida del sebo formando un tapón que se denomina comedón, el cual se compone de queratina, sebo, restos celulares y bacterias, y es la más importante la *P. acnes*; esta última, libera lipasas y proteasas capaces de generar ácidos grasos libres irritantes, además de mediadores proinflamatorios que afectan a la unidad pilosebácea, genera una respuesta inflamatoria y de cuerpo extraño, provoca la aparición de las manifestaciones más comunes del acné: la pápula, la pústula y el nódulo. Los tratamientos más comunes se basan en

antimicrobianos (clindamicina, eritromicina, nadifloxacino, etc.), retinoides (tretinoína, resorcinol, adapaleno, tazaroteno), y otros (ácido azelaico, azufre, peróxido de benzoílo), útiles en el tratamiento del acné inflamatorio (noduloso) y no inflamatorio (comedónico).² No obstante la disponibilidad de numerosos productos de síntesis para enfrentar esta condición, las reacciones adversas a los tratamientos y a la aparición de cepas bacterianas resistentes, obligan a la búsqueda permanente de nuevos agentes terapéuticos, por otra parte, muchos pacientes recurren a terapias alternativas de tratamiento del acné mediante el empleo de productos botánicos y fitoquímicos.³

Dado que el acné inicia con una afección inflamatoria a la que le sobreviene un proceso infeccioso, se evaluaron 3 aceites esenciales (AE) de plantas de uso común en medicina popular para patologías asociadas al dolor, la inflamación, la gastritis, la congestión respiratoria, etc., se abordan los más promisorios, y así poder proponer, formular y evaluar nuevos productos de uso tópico para la prevención/tratamiento del acné como geles y productos cosméticos de aplicación.

Metodos

Recolección del material vegetal

El pericarpio de las frutas de naranja (*C. sinensis*), limón (*C. aurantifolia*) y mandarina (*C. reticulata*) se recolectó en la ciudad de Cartagena, ubicada en el norte del departamento de Bolívar (10°25'25"N 75°31'31"O), Colombia. Se tomaron 1000 g de pericarpio por semana, en el período comprendido de febrero a marzo del 2019.

Procesamiento del material vegetal

El pericarpio de las frutas colectadas se lavó con agua desionizada, y se seleccionó las que se encontraban frescas, enteras, sin señales de deterioro. En seguida se trocearon, pesaron y procesaron inmediatamente.

Procedimientos de extracción del AE

La obtención del AE por HD, se efectuó en un equipo de hidrodestilación del tipo Clevenger 500 g del material vegetal, se introdujeron en el balón de extracción, el cual contenía 500 mL de agua destilada. El tiempo de extracción fue de 3 horas. Por el método MWHD, la obtención se llevó a cabo en un equipo de destilación tipo Clevenger con un reservorio de destilación Dean Stark adaptado a un sistema de calentamiento por radiación de microondas, un horno microondas convencional marca (Samsung, Estados Unidos), con una potencia del 70%, dentro del cual se colocó un balón de extracción de 4 L con 500 mL de agua destilada y 500 g del material vegetal. El tiempo de extracción fue de 3 h⁴⁻⁵. En ambos casos, los aceites esenciales obtenidos se separaron por decantación e inmediatamente se almacenaron en viales

ámbar a 4 °C hasta la realización de los respectivos análisis. Los rendimientos en la extracción se evaluaron por triplicado a tiempos de 20, 30, 40, 50, 60, 90, 120 y 180 min, operando siempre bajo las mismas condiciones, según la ecuación (1):

$$\% \text{ Rendimiento} = (W_{AE} / W_{MV}) * 100$$

Donde, W_{AE} es el peso (g) obtenido del aceite esencial y W_{MV} corresponde al peso en gramos (g) del material vegetal fresco.

Análisis del AE por cromatografía de gases/espectrometría de masas (CG/EM)

Se empleó un equipo CG/EM 7890A/5975C Agilent (Estados Unidos) en interfase con un detector selectivo de masas HP5973 Network conectado en línea con un sistema HP-MS ChemStation y la base de datos NIST-2008. Las condiciones de operación fueron las siguientes: columna capilar HP-5MS (5% phenyl methyl silox, 30 m × 250 µm × 0,25 µm), temperatura inicial 45 °C, temperatura de la línea de transferencia de 280 °C y volumen de inyección 1,0 µL en modo split (20:1), con temperatura del inyector de 250 °C⁵. La detección de los compuestos se realizó por comparación del espectro de masas, en cada tiempo de retención, con los reportados en la base de datos NIST-2008

Diseño de formulación tipo jabón líquido de acción antiséptica y gel con actividad antibacteriana.

Estudio de preformulación

Se realizó un estudio de preformulación para determinar que no existan incompatibilidades entre el principio activo y los auxiliares de formulación, que afecten la estabilidad del producto final, esto se hizo revisando las fichas técnicas de cada materia prima, para verificar las posibles interacciones que existieran entre los componentes y tomar las medidas necesarias. Tablas 1 y 2.

Tabla 1. Formulación del jabón antibacterial a partir de aceite esencial

Componentes	Concentración (%)
Lauril sulfato de sodio	50
Cocoamida	2
Betaina	3
Glicerina	5

Aceite esencial	5
Agua destilada	c.s.p. 100

Tabla 2. Formulación del gel hidroalcohólico antibacterial a partir de aceite esencial

Componentes	Concentración (%)
Carbopol Ultrez 30	0.5
Alcohol etílico 96%	10.5
Glicerina	5
Aceite esencial	5
Agua destilada	c.s.p. 100

Actividad antibacteriana in vitro

La actividad antibacteriana del jabón líquido y gel hidroalcohólico, se realizó de la manera que se describe a continuación.

Cepas de *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 y *P. acnes* ATCC 11827 se inocularon en caldo Tripticasa Soya (TSA), de acuerdo con las indicaciones establecidas por la CLSI, tomando entre 3 y 4 colonias bien diferenciadas y morfológicamente similares de las bacterias previamente sembradas en placas de Petri con agares homólogos, incubándose a 35 ± 2 °C y verificando sistemáticamente la densidad óptica a 620 nm (DO_{620}) en lector de microplacas (Multiscan EX Thermo®, USA), hasta que la suspensión bacteriana alcanzara una DO_{620} entre 0,08 y 0,1 equivalente a 0,5 en la escala de McFarland (1×10^8 UFC/mL)]. Una vez alcanzado este valor, 5 mL de caldo se mezclaron con 5 mL de jabón líquido empleando un agitador Vórtex, en tubos de ensayo microbiológico con tapa estériles, dejándose en incubación por 24 horas a 35 ± 2 °C en agitación constante. Finalizado este tiempo, y para evaluar la capacidad bactericida del jabón

líquido y gel hidroalcohólico, asadas tomadas de los tubos se sembraron en agar TSA y nuevamente se incubaron a las mismas condiciones.

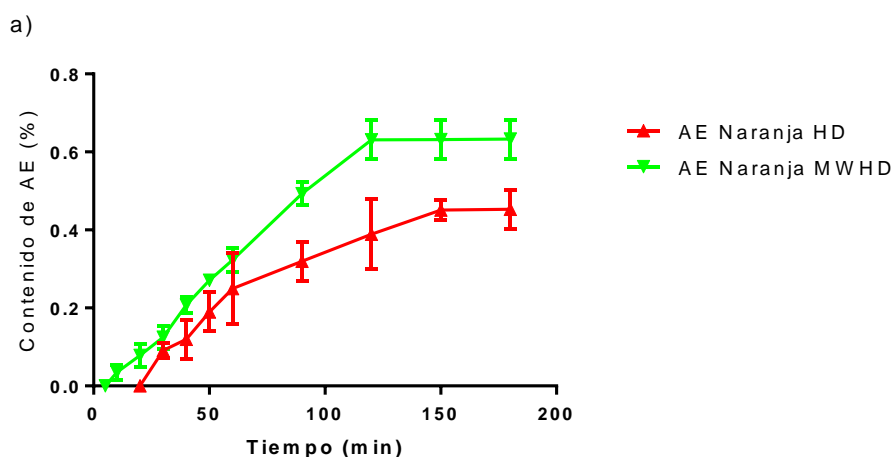
Recolección y procesamiento de la información

Todos los ensayos se realizarán por triplicado. Los resultados se expresarán como la media \pm DE (desviación estándar). Las diferencias significativas se determinarán mediante análisis de T de student o de ANOVA seguido de test de Dunnett o de Tukey, o según el caso. Valores de ** $p < 0,01$ y * $p < 0,05$ contra un control se considerarán significativas.

Resultados

Las plantas aromáticas son fuente de aceites esenciales, productos de alto valor agregado, empleados diariamente como aditivos alimenticios y fragancias, entre otros. El uso de estas plantas, a lo largo de la historia, ha conducido a la búsqueda de métodos de extracción de aceites esenciales que ofrezcan ventajas tanto en la eficiencia de extracción como en la calidad del aceite obtenido. Teniendo en cuenta que la composición química está influenciada por diversos factores, como la temperatura, altitud, condiciones de cultivo, suelo origen y edad de la planta, así como por cambios de tipo genético.

La eficiencia de la extracción de los aceites esenciales de *C. sinensis*, *C. aurantifolia*, *C. reticulata*, se presenta en la figura 1.



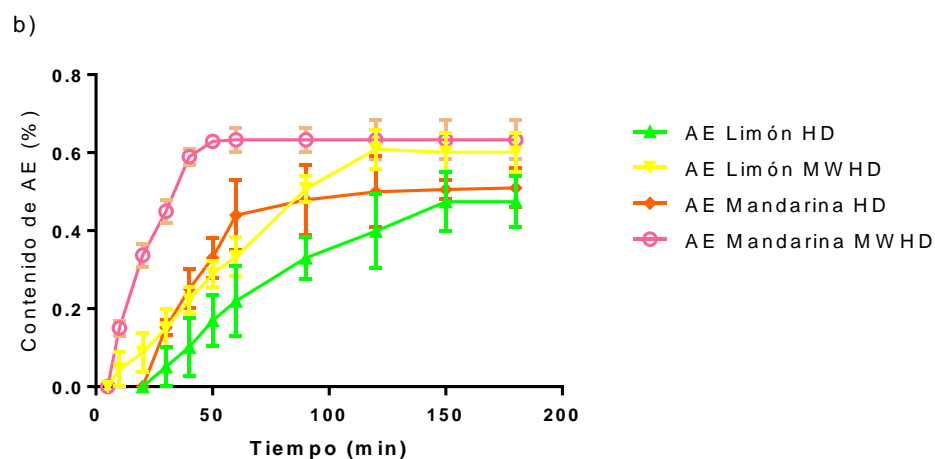


Figura 1. Cinética de extracción de los a) AE de *C. sinensis* b) *C. aurantifolia*, *C. reticulata*, obtenidos por el método de hidrodestilación por arrastre de vapor (HD) e hidrodestilación asistida por microondas (MWHD).

La identificación de los componentes, los tiempos de retención y los porcentajes de abundancia son reportados en la tabla 3. Los monoterpenos son los metabolitos volátiles con mayor abundancia en los AE. El compuesto mayoritario encontrado es el limoneno para ambos métodos "HD y MWHD" en todas las muestras evaluadas.

Tabla 3. Componentes mayoritarios detectados en los AE obtenidos por el método de hidrodestilación por arrastre de vapor (HD) e hidrodestilación asistida por microondas (MWHD).

Compuesto	Porcentaje de abundancia relativa (t_R , min)*					
	<i>C. sinensis</i>		<i>C. aurantifolia</i>		<i>C. reticulata</i>	
	HD	MWHD	HD	MWHD	HD	MWHD
α -pineno	0,38(9,89)**	0,59 (9,90)	1,68(9,09)	1,77(9,00)	0,70(9,91)	0,90(9,91)
β -mirceno	1,17(12,01)	1,39(12,45)	1,00(12,01)	1,09(12,45)	0,90(12,10)	1,00(12,05)
Limoneno	88,80(13,05)	90,20(13,01)	69,28(13,36)	78,80(13,61)	92,22 (13,34)	93,55(13,55)

Linalool	0,60(15,09)	0,89(15,66)	14,60(18,14)	17,89(18,09)	3,03(14,79)	3,89(14,88)
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* Tiempo de retención (t_R) y abundancia relativa (%) de los aceites esenciales, identificados por comparación con espectro de masas de referencia de la base de datos NIST - 2008.

** Todos los resultados presentaron diferencias estadísticas significativas a un nivel de confianza ($P < 0,05$).

Los rendimientos de AE más altos se alcanzaron en las especies de pomelo y mandarina caracterizado por el alto contenido de limoneno. Se encontraron diferencias estadísticas significativas para el rendimiento según el método de extracción empleado. Estos resultados indican que la técnica MWHD mostró ser el método más efectivo en la extracción de los aceites esenciales. Esto es debido a la acción de las microondas sobre las paredes glandulares que contiene el aceite esencial, lo cual hace que el material vegetal se rompa más rápido y eficientemente. La hidrodestilación asistida por microondas utiliza tres formas de transferencia de calor dentro de la muestra: la irradiación, conducción y convección. Como resultado, produce calor con mayor rapidez dentro y fuera de las glándulas. Con la HD esta transferencia de calor solo puede ocurrir por conducción y convección, lo que la hace menos efectiva.

De igual manera, la técnica de MWHD presenta ventajas destacables en comparación con HD en cuanto a disminución de tiempo y uso de solvente (agua), calentamiento efectivo y tamaño de equipo reducido. Sin embargo, la HD es un método sencillo, reproducible, económico y, sobre todo, ampliamente utilizado en la industria. Para determinar si el AE mantiene su actividad antibacteriana al ser incorporado dentro del jabón líquido y gel, se determinó la actividad antibacteriana *in-vitro* del producto terminado, cuyos resultados se presentan en las tablas 4 y 5.



Tabla 4. Actividad antibacteriana del jabón líquido usando como activos aceites esenciales del genero *Citrus*.

Jabón líquido			UFC de <i>S. epidermidis</i>		UFC de <i>S. aureus</i>		UFC de <i>P. acnes</i>	
			t ₀	T ₃₀	t ₀	T ₃₀	t ₀	T ₃₀
<i>C. sinensis</i>	HD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón
	MWHD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón
<i>C. aurantifolia</i>	HD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón
	MWHD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón
<i>C. reticulata</i>	HD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón
	MWHD	5%	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón	0 UFC/mL de Jabón

			Jabón	Jabón	Jabón	Jabón	Jabón	Jabón
Blanco			Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables

Tabla 5. Actividad antibacteriana de gel hidroalcohólico usando como activos aceites esenciales del genero *Citrus*.

Gel hidroalcohólico			UFC de <i>S. epidermidis</i>		UFC de <i>S. aureus</i>		UFC de <i>P. acnes</i>	
			t ₀	T ₃₀	t ₀	T ₃₀	t ₀	T ₃₀
<i>C. sinensis</i>	HD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel
	MWHD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel
C.	HD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel

<i>aurantifolia</i>					gel			
	MWHD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel
C. <i>reticulata</i>	HD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel
	MWHD	5%	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel	0 UFC/mL de gel
Blanco			Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables	Colonias incontables

Posteriormente al desarrollo de un nuevo producto antibacteriano, se determinaron las características fisicoquímicas y químicas (tablas 6, 7, 8 y 9). Esta última se llevó a cabo por GC/MS para estimar composición del AE.

Tabla 6. Componentes mayoritarios detectados en los principios activos utilizadas en el desarrollo del jabón líquido

Compuesto	Porcentaje de abundancia relativa (t_R , min) [*]					
	<i>C. sinensis</i>		<i>C. aurantifolia</i>		<i>C. reticulata</i>	
	HD	MWHD	HD	MWHD	HD	MWHD
α -pineno	0,30(9,09)**	0,50 (9,10)	1,60(9,01)	1,68(9,10)	0,60(9,01)	0,80(9,11)
β -mirreno	1,10(11,21)	1,29(11,22)	0,96(11,77)	1,00(12,01)	0,88(12,00)	0,90(12,01)
Limoneno	88,00(12,85)	90,00(12,51)	69,00(12,56)	77,60(13,01)	91,15 (13,00)	93,00(13,05)
Linalool	0,50(15,12)	0,85(15,33)	13,50(18,05)	16,99(18,11)	2,98(14,11)	3,79(14,33)

* Tiempo de retención (t_R) y abundancia relativa (%) de los aceites esenciales, identificados por comparación con espectro de masas de referencia de la base de datos NIST - 2008.

** Todos los resultados presentaron diferencias estadísticas significativas a un nivel de confianza ($P < 0,05$).

Tabla 7. Componentes mayoritarios detectados en los principios activos utilizados en el desarrollo del gel hidroalcohólico

Compuesto	Porcentaje de abundancia relativa (t_R , min) [*]					
	<i>C. sinensis</i>		<i>C. aurantifolia</i>		<i>C. reticulata</i>	
	HD	MWHD	HD	MWHD	HD	MWHD
α -pineno	0,31(9,11)**	0,49 (9,12)	1,61(9,11)	1,67(9,12)	0,61(9,10)	0,81(9,10)



β -mirceno	1,09(11,20)	1,30(11,12)	0,97(11,70)	1,00(12,00)	0,88(11,59)	0,91(12,11)
Limoneno	88,50(12,75)	90,10(12,33)	69,10(12,76)	77,70(13,11)	91,25 (13,10)	93,10(13,15)
Linalool	0,51(15,22)	0,88(15,43)	13,60(18,12)	17,01(18,22)	2,99(14,12)	3,83(14,12)

* Tiempo de retención (t_R) y abundancia relativa (%) de los aceites esenciales, identificados por comparación con espectro de masas de referencia de la base de datos NIST - 2008.

** Todos los resultados presentaron diferencias estadísticas significativas a un nivel de confianza ($P < 0,05$).

Los perfiles de composición de los principios activos se presentan en las tablas 5 y 6, evidenciándose la presencia los componentes de reconocida actividad antibacteriana como el linalool y limoneno, asimismo no se observa la aparición de nuevas especies químicas, lo que significa que hubo una pequeña pérdida de material en comparación a la concentración inicial de los aceites esenciales, pero no se evidenció deterioro de los componentes constitutivos.



Tabla 8. Propiedades fisicoquímicas de los jabones líquidos antibacteriales

Aceites esenciales usados como activos	Método de extracción	Concentración	Viscosidad (cP)	Color	Olor	pH
<i>C. sinensis</i>	HD	5%	3155,33± 0,99	Blanco	Característico del AE	5,50± 0,13
	MWHD	5%	3245,45 ± 0,25	Blanco	Característico del AE	5,55± 0,15
<i>C. aurantifolia</i>	HD	5%	2545,15 ± 0,33	Blanco	Característico del AE	5,61± 0,19
	MWHD	5%	2645,15 ± 0,55	Blanco	Característico del AE	5,64± 0,75
<i>C. reticulata</i>	HD	5%	3009,22 ± 0,40	Blanco	Característico del AE	5,65± 0,24

	MWHD	5%	3015,23 ± 0,15	Blanco	Característico del AE	5,67± 0,22
Blanco	Blanco		2005,1± 0,25	Blanco	Inoloro	5,8± 0,33

Tabla 9. Propiedades fisicoquímicas de gel hidroalcohólico

Aceites esenciales usados como activos	Método de extracción	Concentración	Viscosidad (cP)	Color	Olor	pH
<i>C. sinensis</i>	HD	5%	4722,08 ± 0,11	Blanco	Característico del AE	6,50± 0,23
	MWHD	5%	4811,12 ± 0,15	Blanco	Característico del AE	6,75± 0,35
<i>C. aurantifolia</i>	HD	5%	4035,15 ± 0,22	Blanco	Característico del AE	6,98± 0,19

	MWHD	5%	4255,09 ± 0,25	Blanco	Característico del AE	7,03± 0,18
<i>C. reticulata</i>	HD	5%	4555,25 ± 0,15	Blanco	Característico del AE	7,3± 0,22
	MWHD	5%	4755,19 ± 0,35	Blanco	Característico del AE	7,43± 0,11
Blanco	Blanco		2005,1± 0,25	Blanco	Inoloro	7,5± 0,22

Generalmente en las preparaciones de aplicación en la piel, el pH debe estar comprendido entre 4,5-7,5 de modo que no se produzca irritación y daño a la piel. El pH de los jabones líquidos antibacteriales y los geles hidroalcohólicos oscilaron en un rango de 5,5-5,8 y 6,5-7,43 respectivamente, los cuales se encuentran dentro de los valores recomendados, lo anterior garantiza que las preparaciones no provoquen irritación en el momento de su aplicación y, además, constituye un indicador de estabilidad física.

En el análisis reológico se observó un comportamiento característico en ambas preparaciones, obteniendo valores comprendidos entre 2545,15 y 3245,45 cP en los jabones líquidos, mientras que para el caso de los geles hidroalcohólicos los valores oscilaron alrededor de 4035,15 y 4811,12 cP observándose que valores de viscosidad son más bajos en el jabón líquido, lo cual es coherente teniendo en cuenta que los geles tiende a poseer una apariencia más compacta comparada con los jabones líquidos. Además, se determinó que las formulaciones corresponden a un fluido no newtoniano con tixotropía positiva. Las propiedades reológicas deben proporcionar al preparado una adecuada extensibilidad y adaptabilidad a la superficie y cavidades cutáneas.

De esta manera se sigue sumando evidencia sosteniendo que los aceites esenciales, son una buena fuente natural y disponible que posibilitará desarrollar diferentes formas farmacéuticas con actividad farmacológica definida. Por otro lado, estos resultados nos pueden servir para comenzar a entender las razones del extenso uso de los aceites esenciales, ya sean en la medicina tradicional o en la aromaterapia, al mismo tiempo podemos acercarnos cada vez más a la utilización de las plantas aromáticas, que contienen aceites esenciales, como terapia complementaria de las convencionales. Como conclusión se puede indicar que los jabones líquidos y geles hidroalcohólicos antibacterianos formulados a partir de un 5% de aceites esenciales del genero *Citrus* presentaron indicadores químicos y microbiológicos promisorios; lo que nos brinda un indicio de la correcta formulación desde el punto de vista galénico.

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Looking for Antifungals: Examination of Natural 1,2,3-Substituted-4-Aryl-Tetralins *N*-myristoyltransferase Inhibitors

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Introduction

1,2,3-substituted-4-aryl-tetralins are naturally-occurring compounds that are present in some species of Magnoliopsida class. Wild species from this clade are widely distributed in our country, but they have been little studied at biochemical and phytochemical level. These plants can synthesize a large number of aromatic compounds derived from the Shiquimate pathway, which exhibited different biological activities, such as antifungal activity. Regarding the problem about fungal diseases worldwide, there is an endless search for more reliable and effective antifungal agents. In this sense, 4-aryl-tetralins-containing compounds can be considered as important moiety for the development of effective antifungals. Thus, as part of our research on some Magnoliids chemistry, a phytochemical study on two Myristicaceae plants was initially performed focusing on separation, identification and structural elucidation of this kind of compounds and its implications on antifungal activity through *N*-myristoyltransferase inhibition.

Method

Extracts were obtained from leaves and bark of two Myristicaceae (*Otoba acuminata* and *Virola macrocarpa*) plants through ultrasound-assisted L-L extraction using ethanol 96%. A portion of crude each resulting extract was separately fractionated and deputed by successive column chromatography (CC) on SiO₂ processes to yield 4-aryl-tetralins **1-11**, following a detailed workflow. Isolated compounds were structurally elucidated using spectrometric/spectroscopic techniques such as MS, ¹H and ¹³C NMR (1D and 2D). Resulting extracts and pure compounds were separately tested against *C. albicans* through a microdilution method at 0.01-5.0 µg/µL in order to calculate the minimum inhibitory concentration (MIC) in µg/mL. Isolated compounds were also tested using an *in-vitro* enzymatic assay for *N*-myristoyltransferase of *Candida albicans* (CaNMT) and the respective half-maximal inhibitory concentration (IC₅₀), in µg/mL, was determined. The binding mode of identified 4-aryl-tetralins within the active site of CaNMT was finally studied through molecular docking using Autodock/Vina and molecular dynamics using Gromacs.

Results/Discussion/Conclusion

Ultrasound-assisted L-L extraction protocol over plant materials resulted in a yield within 8-10% range. CC procedures led to the isolation of eleven 4-aryl-tetralins-like compounds. The present work constitutes the first report of these isolated 4-aryl-tetralins from such plants. MS analyses showed the typical diagnostic peaks for this type of compounds. ^1H NMR and ^{13}C NMR exhibited the typical signals for 4-aryl-tetralins and indicated the presence of 1,2,3-substitutions as a common structural feature of isolated compounds. All compounds exhibited antifungal activity against *C. albicans* at different levels. Aryltetralin **8** showed the best antifungal activity ($\text{MIC} < 0.6 \mu\text{M}$). Good correlations were also found between *in vitro* activities and *in-silico* approach. Most stable conformers of the most active compounds were found to exhibit the best molecular profile and behavior over time, involving crucial interactions with some residues within the active site of the test enzyme. Aryltetralin lignans might be considered as potential leads for antifungal agents development.

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Evaluación del efecto de consumo de suplementos alimenticios que contienen fibra de nopal (*Opuntia ficus-indica*) en personas que presentan sobrepeso y obesidad

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Abstract

El objetivo de este estudio, fue evaluar el efecto de la administración de suplemento a base de nopal deshidratado (*Opuntia ficus-indica*), en pacientes que presentaban sobrepeso y obesidad, mediante un ensayo con asignación al azar y controlado con placebo. Se evaluaron: peso, índice de masa corporal (IMC), colesterol total, triglicéridos, glucosa y hemoglobina glucosilada. Los pacientes que consumieron suplemento a base de nopal (3 gr/día niños y 10-15 gr/ día adultos), disminuyeron significativamente las concentraciones (hasta en un 15 %) de triglicéridos y colesterol. También se observó una tendencia hacia la reducción de las concentraciones de glucosa y presión arterial. Como consecuencia de lo anterior el 80% de los pacientes tuvieron una disminución en el peso corporal.

Anticancer Activity on Human Oral Cancer Cell Lines of Extracts of *Piper mollicomum* Kunth (Piperaceae) from Different Sites of Collection

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Introduction

Piper mollicomum Kunth (Piperaceae) is a native species, popularly known as jaborandi and pariparoba. This species is a shrub and has great distribution in Brazilian territory, occurring from North to South (FLORA DO BRASIL, 2019). *P. mollicomum* has been recognized as a medicinal and aromatic plant since the beginning of the 18th Century. This work aims to investigate the chemical composition and biological activity from extracts of *P. mollicomum* collected at four different sites in the Rio de Janeiro State, Brazil, as well as to correlate the differences in the chemical composition with the biological activity.

Method

Leaves of *Piper mollicomum* Kunth were collected in four sites: I-Rio de Janeiro (PMR, Tijuca Forest National Park, RB 730964); II-Mangaratiba (PMM, Marambaia Island, RB 730967); III-Teresópolis (PMT, Serra dos Órgãos National Park, RB730966); IV-Niterói (PMN, Serra da Tiririca State Park, RB730965). The plant materials were oven dried with 60 °C air circulation and then separately extracted with methanol (1g/ 10 mL) in an ultrasonic bath (Unique USC1400) for 30 min. The methanolic extracts were submitted to analysis by HPLC in a Shimadzu Prominense coupled to a Bruker micrOTOF II High performance mass spectrometer (HPLC-MS/TOF). Compounds identification were achieved by the respective mass spectra obtained with exact mass and comparison with the database MassEuropeanDataBank, as well as with mass records of compounds already described in the literature for *P. mollicomum*. Ionization at 50 eV produced fragments from the molecular ion that aided in the confirmation of the compounds. The human SCC9 and SCC25 cells, derived from a human oral tongue SCC, were obtained from ATCC (CRL-1629 and CRL-1628, respectively). Primary normal human gingival fibroblast was obtained from ATCC (PCS-201-018).

The SCC cell lines and primary human fibroblast cell metabolic activity (viability) was evaluated using the MTT assay. The IC_{50} values for the MTT assay were obtained by nonlinear regression using the GRAPHPAD 5.0 program. Selective index was calculated as $S.I. = IC_{50}$ of plant extract in normal oral fibroblasts cells / IC_{50} of the same plant extract the average IC_{50} of oral cancer cell lines (SCC9 and SCC25).

Results/Discussion/Conclusion

Analysis by HPLC-MS/TOF allowed the identification of chromenes, flavonoids (chalcones, flavones and flavanones) and cinnamic acid derivatives. All extracts were active against OSCC cells but with different potencies and selectiveness. The most selective plant extracts were from PMM and PMR that displayed the largest pharmacological window (SI of 4.13 and 4.04, respectively). On the other hand, extracts from PMN and PMT showed a very similar cytotoxic and selective profile (SI of 2.42 and 2.72, respectively). It can be explained by the chemical composition of the extracts since PMR showed to be very similar to PMM and these two almost like PMN. However, PMR, PMM and PMN were very chemically different to PMT (altitude sample). Our findings support that plants from different collection sites may have different chemical composition that will influence in the biological activity and thus medicinal plants need to be domesticated to be used as herbal medicines.

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Silkworm larvae (*Bombyx mori*) proteins in health

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Introduction

Protein malnutrition in Mexico is a problem that should be examined within the whole context of the national socio-economic milieu. Proteins are an essential macronutrient humans require to have adequate cellular and organ functions. The diet must contain not only enough protein and amino acids, but also sufficient protein energy to permit optimal utilization of dietary proteins. Insects consumption is a cultural tradition for Mexican population, this small animals provide macronutrients, including proteins with all essential amino acids. This study was conducted to assess macronutrient composition of the larvae of *Bombyx mori* silkworm edible insect that can help to improve health.

Method

Silkworms were obtained at Hidalgo State in Mexico, late spring 2018 and *Bombyx mori* larvae, analyzed in dry basis according AOAC (1995) methods to determine, proteins, lipids, total minerals, fiber and soluble carbohydrates.

Results/Discussion/Conclusion

Data obtain was: proteins 52.07%; lipids 29.82%; minerals 5.24%; fiber 0.86%; soluble carbohydrates 12.01%. Protein deficiency has adverse effects in all organs and on particular concern may have long term consequences on brain functions. Silkworm edible insect will represent a valuable source of proteins in a diet, and plays a crucial role for the support of the body physiological and organs systems for a good nutrition and health.

Nutraceutical benefits from chicatana ant consumption in san felipe, otlaltepec, Puebla

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Introduction

Food security is a current international problem, due to constant population increase, demanding for food available, mainly for animal-based protein sources, to satisfy their needs, lead several countries to search different nutritional polices to augment foodstuff to improve welfare of more than 850 million of malnourish people in the world. In San Felipe, Otlaltepec, mixteca region located in Puebla State, people has been neglected the exploitation of other underutilized food sources, with high protein value, such as insects, consumed by cultural tradition every once in a while, but not in a daily diet. Chicatana (*Atta Mexicana B*) adult ants, edible insect consume, by Popoloca ethnic group from the mention region, could provide nutraceutical benefits, to improve their nutrition and welfare. The aim of this study was to assess the nutrient content of Chicatana adult ants, focus in proteins and amino acids and promote their consumption in a regular diet.

Method

Conventional sampling was provided early summer 2018 in San Felipe, and insects analyzed in dry basis, according AOAC (1995) techniques.

Results/Discussion/Conclusion

Data obtain for macronutrients was: moisture 29.75%; proteins 32.87%; lipids 33.30%; minerals 7.78%; fiber 6.33%; soluble carbohydrates 19.72%. For amino acids: mg/16Gn. Ile 5.3; leu 7.8; lys 4.8; met + cys 5.1; phe + tyr 13.2; thr 4.5; trp 0.6; val 6.5, essential amino acids, not synthesized in the body, that has to be consumed in the diet. Proteins are essential in life. The nutritional importance of proteins is as a source of amino acids, their building blocks, also involve in many other functions necessary for physical and mental health of humans. Protein content in insects is high, and low in carbohydrates source of energy, however excess of proteins by the gluconeogenesis process, is converted in glucose, that provide the energy requested for a balance diet. Chicatana ants are a source of nutrients for people of San Felipe, Otlaltepec, Puebla.



Evaluation of the opsonophagocytic index in the burn patient treated with *Aloe vera* L.

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Abstract

This paper presents the results of the behaviour of the opsonophagocytic index in 40 burned patients distributed into 3 strata: serious, very serious and critical condition, and into 2 groups: the first one composed by those treated with *Aloe vera* L. extract plus the ordinary treatment) and the second one made up of those patients receiving the ordinary treatment. Blood extractions were performed at different times (24h, 10th day and 21th day) and phagocytosis was measured at 15 and 60 minutes. During the first 24 hours, the behaviour of both study groups was similar; however, from the 10th day on, a significant improvement of the opsonophagocytic index was observed in the group treated with *Aloe* at 60minutes.

Words key: aloe, burns, immunity cellular, sepsis.





Aplicación de Técnicas Terapéuticas, en el consumo del tabaco

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Introducción

En las últimas décadas, numerosas investigaciones han demostrado la repercusión del tabaquismo en la salud humana, siendo uno de los factores de riesgo modificable más significativo para el desarrollo de diferentes enfermedades presentes en el cuadro de morbi-mortalidad de la población cubana, entre ellas, las enfermedades cerebro-vasculares, cardiovasculares, respiratorias, diversos tipos de cáncer, arteriosclerosis, y otras. Esta realidad hace necesario que se realicen acciones dirigidas al logro de la disminución sistemática del consumo de tabaco en nuestra población.

Objetivo

Demostrar la efectividad de alternativas terapéuticas en la deshabituación tabáquica en un grupo de estudiantes de la carrera de medicina, de la Facultad de Ciencias Médicas Salvador Allende.

Metodología

Se utiliza el método experimental. Previa realización del diagnóstico inicial de los saludables. Al concluir el programa terapéutico se realiza la evaluación final en cada grupo variable a modificar, se aplica durante 4 semanas terapéutica combinada, Auriculoterapia y terapia grupal, con vistas a erradicar o disminuir el consumo de tabaco, y modificar estilos de vida no

Resultados

Se comprueba en el grupo estudiado la disminución del tabaquismo, el aumento de la percepción de riesgo y en la concientización de sus nefastas consecuencias en la salud.

Conclusiones

Resulta efectiva la aplicación de las modalidades, técnicas y herramientas utilizadas en el tratamiento de deshabituación tabáquica en el grupo estudiado, con el consiguiente incremento de estilos de vida saludables en este grupo de futuros profesionales de la salud.



Frases Claves: Auriculoterapia. Deshabitación

Where are we going medicinal plants?

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Medicinal plants have been an integral part of the Chinese, Indian and Arabian ancient culture as medicine and their importance even dates back to the Neanderthal period.

In spite of this, we know little about medicinal plant use by common people in medieval and early modern Europe. In the 18th century knowledge about plant derived drugs expanded, but the attempts to indentify the active ingredients from plants were unsuccessful. In the early 19th century, the term “pharmacognosy” was coined by Johann Adam Schmid (1759-1809), but the main shift came when it became clear that the pharmaceutical properties of plants are due to specific molecules that can be isolated and characterized.

The 20th century saw the integration of ethnobotanical, pharmacological and phytochemical studies, a process that had taken many and many years, but which allowed the development of a new approach to the study of the pharmaceutical use of plants.

Ultimately, herbal remedies and natural products are transformed into chemically defined drugs. These offer large structural diversity, modern techniques for separation, structure elucidation, screening and combinatorial synthesis, have lead to revitalization of plant products as a source of new drugs. This has opened up new opportunity and avenues for drug development.

Furthermore, the introduction of plants in the form of nutraceuticals and dietary supplements are also changing the plant-based drug market.

Lastly, it is important to carry out surveillance on medicinal plants, in fact many products are put on the market without any control.

In conclusion, the use of medicinal plants is still an important part of the medicinal systems all over the world, without exception for any country



Intervención Educativa en adolescentes de S/B para promover la prevención del consumo de sustancias adictivas

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La experimentación y el inicio del consumo de sustancias adictivas se producen típicamente en los primeros años de la adolescencia. Para trabajar en la prevención de estas sustancias nos proponemos a realizar una investigación descriptiva longitudinal en dos centros estudiantiles aledaños a la facultad, secundarias básicas del municipio Cerro. Con el objetivo de determinar el conocimiento, actitud y percepción de riesgo que poseen los adolescentes para el diseño de un programa educativo y la formación de una red de promotores adolescentes que promuevan los efectos negativos que para la salud trae el consumo de estas sustancias.

Para esto se informará las características del estudio a la dirección de los centros, se tendrá el consentimiento de padres y se aplicará una encuesta en adolescentes que recoja variables sociodemográficas, conocimiento sobre el alcohol y tabaco, actitud ante el consumo, percepción de riesgo y voluntariedad para capacitarse como promotores.

La formación de estudiantes adolescentes voluntarios que se identifiquen con la problemática y que estén dispuestos a servir de promotores para la salud, facilitaría la introducción de información, conductas y actitudes a favor de la prevención del consumo de estas sustancias adictivas.

Los resultados se procesarán utilizando el SSPS 15 para obtener medidas de frecuencia y resumen adecuados que caractericen las variables seleccionadas, estos se mostrarán en tablas y gráficos.

PLANTEAMIENTO DEL PROBLEMA

¿Qué conocimientos poseen los adolescentes de edad temprana con relación a la percepción de riesgo y actitudes negativas con relación al consumo de Sustancias Adictivas?

Determination of antifungal efficiency of the aqueous extract of Sospó flower (*Pseudobombax ellipticum*) in the fungi *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans*.

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Introduction

The suspó flower is a species native to Mexico, its reproduction has been distributed throughout a large variety of tropical regions of America as a medicinal plant, in some regions it is used to relieve respiratory diseases and stomach infections, in this project it is sought to analyze its effect on different fungi: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans*. which are the cause of diseases such as airway obstruction, candidiasis, invasive pulmonary aspergillosis, sinusitis, among others, and which, when treated with commercial antimicrobics, can frequently be harmful to health.

Method

From the CLSI method, a sensitivity analysis was performed for the fungi *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* using aqueous solutions at six different concentrations of 100, 200, 400, 600, 800 and 1000 $\mu\text{L mL}^{-1}$ of the flower of suspó. The fungi were inoculated respectively in triplicate on PDA agar (potato dextrose, agar) and then the sensidiscs were placed, taking into account a control (+) Voriconazole and a control (-) water, once this process was done, the fungus was left to incubate at 35 ° for 5-7 days, the halo was subsequently read by measuring the inhibition halo, making the susceptible or resistant classification depending on the results obtained.

Results/Discussion/Conclusion

Retaking antecedents it is estimated that the results are favorable in average concentrations between 400 and 600 $\mu\text{L mL}^{-1}$ of the flower of suspect since in tests made of it but in its leaves and taking as ethanol solvent, they show that both *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*) and *Candida albicans* are sensitive to these concentrations, however the possibility that the result varies due to the different factors that intervene at the time of testing which can be from the flower to the solvent used is not ruled out. in this case water.

Analyzing the results obtained *Aspergillus flavus* and *niger* show greater sensitivity to the different concentrations presented, whereas in the case of *Candida albicans* and *Aspergillus fumigatus* they show a high resistance with the exception of the solutions of 400 and 600 $\mu\text{L mL}^{-1}$ where the halos of sensitivity were classified with medium susceptibility. So it is determined that the flower of Sospó (*Pseudobombax ellipticum*) is a candidate for antifungal.

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Análisis del aceite esencial de hojas de *Salmea scandens*, una especie con potencial antimicrobiano y antiinflamatorio

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Introducción

Salmea scandens DC. (Asteraceae) es una especie de uso medicinal en Oaxaca, México. Las hojas y corteza son usadas en la medicina tradicional en América por sus propiedades analgésicas, anestésicas, antipiréticas, antidiarreicas y antimicrobianas [1]. En Oaxaca, las hojas son utilizadas para aliviar el “clavillo”, un padecimiento que se caracteriza por la aparición de un forúnculo. Se usan hojas frescas en forma de caplasma. Investigaciones sobre los componentes químicos bioactivos de las hojas de *S. scandens* ayudarían a reafirmar su uso en la medicina tradicional en Oaxaca. En el presente trabajo investigamos la composición de volátiles y una búsqueda de los posibles componentes bioactivos de las hojas de *S. scandens* nativa de Oaxaca.

Material y métodos

Material vegetal. *Salmea scandens* fue colectada en septiembre 2018, coordenadas 16° 07' 12" Norte y 96° 18' 57" Oeste, entre 2359-2380 msnm. En San Andrés Paxtlán, Miahuatlán, Oaxaca, México. El ejemplar fue depositado en el herbáreo del Instituto Politécnico Nacional, CIIDIR, Oaxaca. La especie fue identificada por la Prof. Rufina García.

Obtención de aceite esencial. 200 g de hojas frescas fueron pulverizadas y sometidas a hidrodestilación asistida con micro-ondas a 2.45 MHz por 60 min. El aceite esencial fue secado con Na₂SO₄ anhidro y almacenado en frasco ambar a 4 °C hasta su análisis.

Análisis por GC-MS. La composición del aceite esencial fue analizada por GC-MS usando un EVOQ GC-TQ, Bruker. El método de adquisición fue full-scan. La identificación de los componentes se realizó por comparación de los espectros de masa con la base de datos NIST y los índices de retención reportados en la literatura [2].

Resultados y Discusión

Treinta y seis componentes se identificaron en el aceite esencial de hojas de *S. scandens*. El contenido de sesquiterpenos fue claramente abundante siendo germacreno D (21.9%) el mayoritario. Los resultados muestran 31 componentes por primera vez identificados en el aceite esencial de hojas de esta planta. Trabajos previos identificaron los componentes germacreno D, escualeno, nerolidol, espatulenol en las partes aéreas de *S. scandens* [3]. Nuestro análisis del aceite esencial de hojas contiene los componentes antes mencionados, con excepción del escualeno. Al menos tres de los componentes identificados en el aceite esencial de hojas (cadinol, β -cariofileno y α -copaeno) se ha reportado su potencial antimicrobiano y antiinflamatorio. Estos resultados proporcionan un respaldo científico del efecto farmacológico de las hojas de esta planta en la medicina tradicional.

Conclusión

Se coadyuba al fortalecimiento del uso medicinal de esta planta en el estado de Oaxaca, al identificar al menos tres metabolitos secundarios en el aceite esencial de hojas de *S. scandens* con propiedades antimicrobianas y antiinflamatorias. Asimismo, se contribuye al conocimiento de su fitoquímica.

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Isoflavonoids in soybean sprouts treated with elicitors

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Introduction

Soybean sprouts are natural source of nutrients with beneficial effects for health; their consumption reduce the risk to suffer cardiovascular diseases and cancer. During soybean germination occurs metabolic changes that affect the accumulation of isoflavones; bioactive compounds with phytoestrogen activity that prevents some important diseases, such as osteoporosis and cancer. These compounds are found in soybean as glucosides (daidzin, 6-*O*'-malonyl-daidzin, genistin, 6-*O*'-malonyl-genistin) and aglycones (daidzein and genistein); these last one are the most bioactive and have highest absorption in organism. In addition, coumestrol and glyceollins are present in soybean plants and display estrogenic and anticancer properties. In recent year, there is an interest by the increasing of bioactive compound contents through use of elicitors; compounds that stimulate the natural defense mechanism of plants, including biosynthesis and accumulation of secondary metabolites. In order to identify soybean cultivars with high content of isoflavones, daidzin, 6-*O*'-malonyl-daidzin, daidzein, genistin, 6-*O*'-malonyl-genistin, genistein, coumestrol and glyceollins were quantified in sprouts soybean (cultivars Panorama 29, Panorama 357, Panorama 358, Soyica P34 and Soyica P27) cultivated in Colombia; also, the effect of storage time on the isoflavonoids concentration was established. Additionally, the content of isoflavones, coumestrol and glyceollins in the sprouts soybean with exogenous application of different elicitors was evaluated.

Method

The elicitors (benzoic acid, methyl jasmonate and 2-aminonicotinic acid) were obtain from Alfa-Aesar, and the compound 1-oxo-indanoyl-(L)-isoleucine methyl ester was prepared from 2-carboxy-hydroxycinnamic through different reactions. The seeds of five varieties of soybeans were allowed to germinate for 7 days until obtaining seedlings. Then, these were treated with each elicitor (1.6 mM) for 4 hours, untreated seedlings were used as a negative control. After induction, the seedlings were again sown and the isoflavonoid extracts obtained at different times (0, 24, 48 and 72 h). The seedlings (7 g) were extracted by maceration with ethanol; after filtration, the solvent was evaporated and the residue dissolved with ethyl acetate. The content of isoflavones in the extract was determined by HPLC.

Results

The varieties Soyica P34, Panorama 357 and Panorama 358, after 48 hours of planting, exhibited the highest content of isoflavones, coumestrol and glyceollins. On the other hand, in all varieties of soybean the elicited seedlings showed maximum accumulation of compounds (daidzin, 6-O"-malonyl-daidzin, daidzeine, genistin, 6-O"-malonyl-genistin, genisteine, coumestrol and glyceollins) between 24 and 48 h.

Conclusion

The contents of isoflavonoids in soybean seedlings were influenced by the variety, the type of elicitor and the time after induction. These results offer an alternative in the future to improve the levels of isoflavones, coumestrol and glyceollins in soybean.

Sinapis alba* L. antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa

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Introduction

Sinapis alba L is a plant belonging to the family Cruciferae or Brassicaceae. Its Latin genus "sinapis" which means mustard, and the Latin species "alba" which means white, refers to the light color of its seeds. It is widely recognized as the producer of mustard, however, in addition to its culinary use has medicinal advantages. Its seeds and oil are consumed as palliative treatments for cancer, bronchitis, pneumonia, among other diseases. In addition, it is believed that the plant has emollient and sedative properties, even narcotic. The antibacterial effect of the essential oils of *Sinapis alba* L is also known, since these contain mainly allyl isothiocyanate, which contains an inhibitory effect on a great variety of bacteria such as *E. coli*, *P. fluorescens*, *S. aureus*, *Bacillus subtilis*, *L. brevis*, *K. pneumoniae*, *P. aeruginosa*.

Method

The microorganisms used were obtained from the bacterial strain collection of the Pontificia Universidad Javeriana. The assembly of the experiment was carried out facing each part of the plant with each of the strains, so 52 g/L of BHI agar was prepared. A suspension of each microorganism was made in BHI broth from each strain, this inoculum had a concentration of 1×10^8 CFU / mL with reference to pattern 1 of McFarland. Finally, for the control of the inoculum, confirmation of the initial concentration was made by plate count on BHI agar. The extraction process of the plant was carried out in the same way for the four parts of the plant: stem, root, leaves and flowers. A sample was taken which was introduced in a thimble, then the Soxhlet extraction process was started using first as petroleum ether solvent, after finishing the process and collecting the extract, the sample was allowed to dry to repeat the Soxhlet extraction with ethanol and finally with dichloromethane, the extracts were finally obtained with a pasty consistency. From the previously prepared inocula, each was mixed separately with the BHI broth. In each 100 x 15 mm petri dish approximately 27 mL of inoculated medium was served, then allowed to solidify under refrigeration. Subsequently, to each agar plate two central perforations were made with an inverted pipette of 10 mL. Finally, 100 μ L of each extract was added in triplicate. This procedure was repeated in the same way for the 3 microorganisms under study. All microorganisms were incubated at 37 ° C for 3 days. Growth control of

each bacterium in the culture medium without perforation was carried out. To perform the blank control, 100 μ L of dimethylsulfoxide (DMSO) was used in duplicate, and a commercial antibacterial was used as a positive control (5.25% sodium hypochlorite). After the incubation, the measurement of the halos of each assembly was carried out and the relative antimicrobial activity with respect to the commercial antibacterial and DMSO was calculated.

Results/Discussion/Conclusion

From the procedure carried out in the practical stage of this work of degree, the results for *E. coli* were obtained, in the Table 2 it can be shown that they did not present inhibition halos with respect to the total extracts of root, stems, leaves and flowers with none of the extraction solvents. In relation to the controls, haloes of inhibition of 5 mm with sodium hypochlorite and 2 mm with DMSO were evidenced. The results of the inhibition of the plant on *P. aeruginosa* shown that the microorganism was resistant to the effect of the plant. Additionally, *P. aeruginosa*, where there was growth inhibition only in the control with sodium hypochlorite. For *S. aureus*, the percentages of inhibition shown that the extract that had the highest percentage of inhibition was the stem with petroleum ether in 5.3%, followed by root with petroleum ether with 3.9%, leaves with petroleum ether and flowers with dichloromethane with 3.8%; in the cases of root with petroleum ether, root with ethanol and leaves with dichloromethane there was no inhibition. In turn, halos of inhibition were presented against the control with sodium hypochlorite, this being 5 mm and for the control with DMSO of 2 mm. For the solvent, the Petroleum ether shows a greater average growth in the diameter of the haloes. Regarding the extracts used, it was determined by the percentage of inhibition that the extract with the highest antibacterial spectrum on *S. aureus*, was the stem extract with petroleum ether, followed by the root extract with petroleum ether, leaves with ether of oil and flowers with dichloromethane. With the test, it was also possible to show that based on the extract, stem and root had a higher percentage of inhibition in all cases and that, in turn, dichloromethane also had better results in comparison with the other solvents.

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Quantification of metals in soils of the high and medium basin of Rio Bogotá

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Introduction

The high and middle watersheds of the Bogotá River have historically suffered the deterioration of ecological systems and geomorphological alterations caused mainly by anthropic processes, due to industrial, agricultural activities and the dumping of all types of waste for decades along the river.

Objectives

To quantify the accumulation of heavy metals (Arsenic, Cadmium, Cobalt, Copper, Chromium, Nickel, Manganese, Lead and Zinc) in the soils of the Bogotá River from its (upper basin): Villapinzón, Chocontá, Chia, Sibaté and Mesitas del Colegio (Middle Basin)

Methodology

Acid digestion was performed on the collected samples with subsequent evaluation of the heavy metals by atomic absorption.

Results and conclusions

With respect to the normal ranges of metals in soils (ppm), As <5-40, Cd <1-2, Cu 2-60, Ni 2-100, Pb 10-150, Zn 25-200 Soils present high contents of the metals products of the discharges to the length of the river Bogotá

mg metal / Kg soil	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Co	Cd	As
Villapinzón	10.24	1.92	1,097.3	110.74	1.34	5.43	26.45	9.45	6.29	22.71
Chocontá	78.97	1.92	1,042.5	64.71	3.34	6.05	43.50	11.55	6.90	24.71
Chía	21.18	1.83	1,120.1	178.09	6.68	12.74	121.57	11.65	6.90	25.22

Sibaté	26.26	1.88	1,009.5	79.91	8.59	9.60	73.63	9.62	6.30	26.12
Mesitas del Colegio	9.60	1.86	1,102.1	117.75	32.48	25.05	217.01	25.76	9.55	29.86
Percentage	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Co	Cd	As
Villapinzón	7.00	20.43	20.43	20.09	2.56	9.22	5.49	13.89	17.50	17.66
Chocontá	54.00	20.45	19.41	11.74	6.37	10.28	9.02	16.98	19.20	19.21
Chía	14.48	19.44	20.85	32.31	12.74	21.63	25.21	17.12	19.20	19.61
Sibaté	17.96	19.94	18.79	14.50	16.39	16.31	15.27	14.14	17.53	20.30
Mesitas del Colegio	6.56	19.75	20.52	21.36	61.95	42.55	45.01	37.86	26.57	23.21

Keywords: Rio Bogotá, Metals

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Assessment of antimicrobial activity of extracts of *Diplostephium phyllicoides* and *Diplostephium revolutum* by the method of plates and wells

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Introduction

Nosocomial infections represent a risk for patients who remain in hospitals, being one of the main public health problems reported in the world. Plants continue to present opportunities for the treatment of diseases caused by microorganisms, since they not only provide benefits in terms of treatment, prevention and cure of diseases, but also provide vitamins, minerals and amino acids to patients who consume them. The present study evaluated the antimicrobial activity of leaf extracts of *Diplostephium phyllicoides* and *Diplostephium revolutum* against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aereas*, *Candida albicans*, and *Saccharomyces cerevisiae*

Method

The plants used in the study were collected along the road between Bogotá and Guasca in a place known as the *páramo de Chingaza* (Cundinamarca, Colombia). The extraction process was carried out with the leaves of each plant separately, using Soxhlet extraction and using as solvents petroleum ether, dichloromethane, ethyl acetate and ethanol; these extracts were solubilized and taken to a concentration of 100 mg / mL in dimethyl sulfoxide (DMSO). Inoculum were made for each microorganism in sterile peptone water, which were added together with Nutritive agar or PDA in Petri dishes.

5 perforations were made in the agar and extracts were added in 4 wells in different volumes (10 µL, 20 µL, 30 µL and 50 µL) and in the last well 20 µL of the positive control (antibacterial or commercial antifungal) was added. DMSO was used as a negative control. All experiments were carried out in triplicate.

For the purpose of determining the antimicrobial activity, the formula of the relative inhibition percentage was used and an analysis of variance (ANOVA) to determine significant results.

Results/Discussion/Conclusion

The findings showed that ethyl acetate extract from *Diplostephium phyllicoides* has significant antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*; additionally, dichloromethane extract showed inhibitory effect against *Saccharomyces cerevisiae*. When comparing the extracts of the two plants, the extract with the highest antimicrobial activity was the ethyl acetate of *Diplostephium revolutum* that showed activity against all the microorganisms evaluated. The antimicrobial activity of *D. phyllicoides* and *D. revolutum* is attributed to the presence of triterpenes (uvaol, bauerenol acetate, friedelin) and flavonoids (genkwanine, quercetin, sorbifolin), which alter the permeability of the lipid bilayer in the plasma membrane and cell wall of the microorganisms evaluated.

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Effects of obex in overweight and obese subjects with or without impaired fasting glucose: a pilot study

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Abstract

Obex is a dietary supplement to help weight loss. The purpose of this study was to evaluate the effect of Obex in overweight/obese participants with or without impaired fasting glucose. This was an openlabel pilot study conducted with 40 overweight and obese subjects, 23– 60 years old with a body mass index of 25–44 kg/m² (20 participants with impaired fasting glucose [IFG] and 20 with normal glucose levels). Participants received Obex at a dose of one sachet before the two main meals of each day for 3 months. In addition to anthropometric measures and blood pressure (BP), fasting plasma glucose, lipid profile, insulin, creatinine, and uric acid were determined. Insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) were assessed. Three indirect indices were used to calculate insulin sensitivity. Compared to baseline, Obex significantly reduced body weight, body mass index, waist circumference, waist/hip ratio, and waist/height ratio in both groups of participants ($p < 0.05$). In individuals without IFG, Obex improved HDL-c (high-density lipoprotein cholesterol) ($p < 0.0001$) and lowered BP ($p < 0.05$). After 3 months of Obex, subjects with IFG showed a reduction in fasting glucose concentrations ($p < 0.0001$). Compared to baseline, this group also showed improved insulin sensitivity and HDL-c ($p < 0.05$). In conclusion, the consumption of Obex contributed to weight reduction, improved glucose tolerance and insulin sensitivity, as well as HDL-c, and appears to be safe in overweight/obese adults with impaired fasting glucose. Obex may be beneficial for weight loss, indicating that further studies are required.

Evaluation of the antimicrobial activity of *Rosmarinus officinalis* and *Eucalyptus globulus* essential oils from Perú

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Objective/Purpose

The antimicrobial activity of the essential oil obtained from the species *Rosmarinus officinalis* and *Eucalyptus globulus* of peruvian origin were evaluated against a battery of seven microorganisms.

Material and Methods: Fresh leaves of *Rosmarinus officinalis* from Tarma and *Eucalyptus globulus* from Huancayo were used in this study. They were extracted their essential oils by hydrodistillation type Clevenger and evaluated the antimicrobial activity of each essential oil by diameter of inhibition of microbial growth, diffusion disk method in agar. Seven microorganisms were evaluated: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* wild, *Clostridium perfringens* ATCC 13124, *Escherichia coli* ATCC 25992, *Salmonella choleraesuis* ATCC 14028 and yeast *Candida albicans* wild. The bacterial strains were cultured by 24 h at 36 ± 1 ° C in nutrient broth while *Candida albicans* at 37 ± 1 ° C.

Results

Regarding the essential oil of the *Rosmarinus officinalis* plant cultivated in the Andean region, a high antimicrobial activity was revealed against the microorganism *Clostridium perfringens*, a moderate one against the *Escherichia coli* and no activity against *S. aureus*, *B. cereus*, *S. choleraeuis*, *L. monocytogenes* and *C. albicans*. In relation to the essential oil of *Eucalyptus globulus*, there was a low antimicrobial activity against *S. aureus*, *L. monocytogenes*, *C. perfringens* and *S. choleraeuis* and null against *B. cereus*, *E. coli* and *C. albicans*. With regard to the controls used, Gentamicin and Amoxicillin, only the oil of *Rosmarinus officinalis* managed to overcome the spectrum of inhibition of Gentamicin against *Clostridium perfringens* while the essential oil of *Eucalyptus globulus* could not overcome the spectrum of inhibition of any control.

Conclusion/Discussion

The results conclude that the essential oil of *Rosmarinus officinalis* presents better antimicrobial activity against *Clostridium perfringens* than its synthetic substitute Gentamicin and better spectrum of inhibition



against E.coli with Amoxicillin. In the case of the essential oil of *Eucalyptus globulus*, all the microorganisms evaluated have a limited sensitivity.

Keywords: *Rosmarinus officinalis*, *Eucalyptus globulus*, antimicrobial activity

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Chemical Diversity in Essential Oils from Leaves of Four *Piper* species from High Altitude Area in Southeastern - Brazil

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Introduction

The Piperaceae family is considered to be one of the most basal classes among the angiosperms [1] with approximately 2000 species in 5 genera (*Manekia* Trel., *Peperomia* Ruiz & Pav., *Piper* L., *Verhuellia* Miq. and *Zippelia* Blume), some of them distributed from North to South of the Brazil. *Piper* is the greatest genus of this family. Research related to the biological activities of essential oils (EO) of *Piper* species has revealed important actions, ranging from antimicrobial, acaricidal, larvicidal, antitumor and insecticidal [2]. It is known that the metabolites present EO modify qualitatively and quantitatively according to variations from biotic and abiotic factors (3). Regions of high altitudes present ecological patterns and stresses quite singular. Serra dos Órgãos National Park (PARNASO) is one of the few areas of tropical climate of altitude that show more important biomasses and critics for the conservation of the global biodiversity [4]. It presents a significant diversity of *Piper* species [5]. Therefore, it is aimed to study the chemical composition of EO from leaves of *Piper aduncum* L., *P. lhotzkyanum* Kunth, *P. mollicomum* Kunth. and *P. truncatum* Vell. collected in PARNASO in the State of Rio de Janeiro - Southeast, Brazil.

Methods

Fresh leaves (150g) of four *Piper* species were extracted by hydrodistillation for three hours in a modified Clevenger-type apparatus to obtain the EO. Compounds identification and quantification were done by Gas Chromatography coupled to Mass Spectrometry (GC-MS) and GC coupled to the Flame Ionization Detector (GC-FID). The retention index was calculated from a homologous series of hydrocarbons (C₈-C₂₆, Sigma-Aldrich) to achieve compounds identification.

Results/Discussion/Conclusion

The results showed a majority fraction characterized by sesquiterpenes, with major compounds recorded as: *P. aduncum*: *E*-caryophyllene - 15.1%, *E*-nerolidol - 10.1%, δ -elemene - 6.2% and bicyclogermacrene - 4.7%; *P. lhotzkyanum*: δ -elemeno - 7.53%, β -elemeno - 33.69% and γ -elemeno - 27.87%; *P. mollicomum*: α -eudesmol - 9.6%, *E*-nerolidol - 12.9% and β -elemene - 9.3%; and *P. truncatum*: 2*E*, 6*Z*-farnesol - 15.3%, 2*E*, 6*E*-farnesol - 7.9% and β -ylangene - 8.9%. It was registered the chemical diversity and production

pattern of sesquiterpenes for the *Piper* species of this high-altitude area. It is known that many of these terpenoids identified in these species as majorities already possess several biological activities, such as antiparasitic, antimicrobial and antitumor activities [1, 2]. Considering that these EO from fresh leaves showed a significant intra-specimen chemical diversity in sesquiterpenes, that can be correlated with this variation of ecological pressures of this altitude area. They are promising for future biological tests.

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Antioxidant and antifungal activity of *Usnea*

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Introduction

Lichens are complex organisms that are derived from a symbiosis between fungi and algae, have the ability to interact in such a way that generate the production of various secondary metabolites for their development and defense, among which are found phenolic compounds which present diverse biological activities.

Objectives

To evaluate the antioxidant activity by the ABTS^{*+} and antifungal method of the total ethanolic extract and fractions of different polarities and to determine its antifungal activity against *Penicillium digitatum*, *Aspergillus niger* and *Rhizopus nigricans*.

Methodology

The total ethanolic extract was obtained which fractionated liquid / liquid are solvents of increasing polarity Hexane, Chloroform and Ethyl Acetoate With subsequent evaluation of the ABTS * + antioxidant capacity and antifungal by the plate drilling technique.

Results and Conclusions

Of *Usnea* varieties, we found that the fraction with the best antioxidant activity is acetone 58.53 mg/L MeOH and the relative antioxidant activity (AAR) with respect to ascorbic acid, trolox and rutin equals 32.23 mg/L MeOH, 25.93 mg/L MeOH and 13.75 mg/L MeOH respectively. For the ABTS^{*+} test, most extracts and fractions showed higher IC₅₀ values, for the Petrol fraction the IC₅₀ presents a value of 144.44 mg/L MeOH, the dichloromethane fraction a value of 114.43 mg/L MeOH, the acetone fraction with a value of 58.53 mg/L MeOH, the ethanolic fraction a value of 68.23 mg/L MeOH and the total ethanolic extract with a value of 163.58 mg/L MeOH. Where the fractions that showed the best activity were the acetone fraction and the ethanolic fraction. With respect to the antifungal activity, we found that the fractions that had better IC₅₀ against the fungus *P. digitatum* was the ethanol fraction (44.33), for *R. nigricans* was the Petrol fraction (75.35) and for *A. niger* was the total extract (35.48). We found that in the antifungal activity related to fluconazole, for the three fungi the dichloromethane fraction has the best activity. The sensitivity obtained with 1 mg of fluconazole against *A. niger* is equivalent to 310.93 mg of the dichloromethane fraction,



compared to *P. digitatum* is equivalent to 222.15 mg of the dichloromethane fraction, and against *R. nigricans* is equivalent to 98.71 mg of the dichloromethane fraction. For the relative antifungal activity with ketoconazole, the dichloromethane fraction has the best activity, the sensitivity obtained from ketoconazole 1 mg for *A. niger* is equivalent to 352.39 mg, for *P. digitatum* it is equivalent to 2383.71 mg and finally in *R. nigricans* it is equivalent to 140.97 mg of the fraction. The extracts of different polarity obtained from the *Usnea* varieties. They have inhibitory activity against the growth of the fungi *A. niger*, *P. digitatum* and *R. nigricans* and present antioxidant activity by ABTS^{*+} method

Key words: *Usnea*, Antimicrobial activity, Antioxidant activity, ABTS^{*+}

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In vitro antimicrobial effect of the hydroetanolic extracts of five medicinal plants in northern Peru on microorganisms of stomatological importance

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Introduction

Dental caries and periodontal disease are oral diseases with high prevalence worldwide. The control of causative agents is mainly chemical. The indiscriminate use of antimicrobials and other factors has led to the manifestation of resistance in microorganisms making them a global public health problem. Medicinal plants have always been a potential source of new drugs in societies. In the northern region of Peru, more than 50 medicinal plants have been described, whose antimicrobial properties on oral microorganisms have not yet been fully studied.

Objectives

To evaluate the in vitro antimicrobial potential of different concentrations of the hydroalcoholic extracts of *Prosopis pallida* (Algarrobo), *Duranta mutisii* (Cashaquiro), *Ruta graveolens* (rue), *Plantago major* (Llantén) and *Cinchona officinalis* (Cascarilla) on *Candida albicans* ATCC 10231, *Streptococcus mutans* ATCC 35668 and *Porphyromonas gingivalis* ATCC 33277.

Material and methods

Total extracts were obtained by hydroetanolic maceration. Bacterial inoculums were standardized to $1,5 \times 10^8$ CFU / mL. Different concentrations in $\mu\text{g} / \text{mL}$ of the extracts of *Prosopis pallida* (Algarrobo), *Duranta mutisii* (Cashaquiro), *Ruta graveolens* (rue), *Plantago major* (Llantén) and *Cinchona officinalis* (Cascarilla), positive antibacterial control of chlorhexidine Gluconate 0.12% and an antifungal control of Nyistatin 100,000 IU were evaluated. The antimicrobial potential was evaluated by discodiffusion and microdilution methods.

Results

The results indicate that the hydroalcoholic extract of all the plants evaluated had antimicrobial potential on certified microbial strains of stomatological interest.



Conclusions

The different concentrations of the tested extracts have an in vitro antimicrobial effect of bactericidal type on *S. mutans* ATCC 25175 and *P. gingivalis* ATCC 33277 and fungicidal type on *C. albicans* ATCC 10231. This effect was statistically significant with respect to controls.

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In vitro analysis of the association of antimicrobial agents with (+) - α -pinene against *Staphylococcus aureus*

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Introduction

Bacterial resistance is a major global health challenge, affecting both developed and emerging countries, as there is a significant increase in the number of bacterial strains that are increasingly resistant to currently available antibiotic therapy. An alternative to this problem is the advancement of studies on herbal medicines, which discusses the use of plants as natural sources of new compounds that have the potential to fight bacterial infections. The objective of this research was to evaluate the effect of the association of (+) - α -pinene with commercial antimicrobials that act on the bacterial cell wall against strains of *Staphylococcus aureus*.

Method

The antimicrobial agents that act on the bacterial cell wall: cefepime, ceftazidime, oxacillin, penicillin and vancomycin acquired from the laboratory Cecon® (São Paulo - SP) were used. The tests were performed on the *Staphylococcus aureus* strain ATCC (American Type Culture Collection) 25923. For this the modified disc-diffusion method was used. Commercial antimicrobial (ATB) discs were applied to the surface of the medium and 20 μ L of the (+) - α -pinene (+ AP) MIC was previously added. The diameters of the halos formed by each association + AP - ATM were compared to those determined by ATM alone. A synergistic effect was considered if the increase in inhibition halo diameter ≥ 2 mm; antagonistic effect, increased diameter smaller than that of isolated TMJ; and indifferent effect when the increase in diameter < 2 mm. All experiments were performed in triplicate and the results analyzed by GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Results/Discussion/Conclusion

The following growth-inhibiting halo diameters were found: (+) - α -pinene: 14.33 mm, cefepime: 30.00 mm, oxacillin: 27.67 mm, penicillin G: 41.00 mm, vancomycin : 20.67 mm and ceftazidime: 31.33 mm. After the association, the diameter values of growth inhibition halos were changed to: cefepime: 31.67 mm, oxacillin: 34.33 mm, penicillin G: 39.33 mm, vancomycin: 24.00 mm and ceftazidime: 32.67 mm. After applying the

statistical analysis, it was concluded that the association of (+) - α -pinene with the antimicrobials did not present significant modification in the diameter of the halos of inhibition of growth. In this way, the association test is necessary for other drugs with different mechanisms of action.

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Vascular activity of *Cymbopogon citratus* leaf tincture

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Introduction: *Cymbopogon citratus* used in traditional medicine. It has been shown to be effective in the treatment of infection, headaches, and rheumatic pain. It is also reported to act as a sedative, antispasmodic, and antihypertensive agent (Kouame et al., 2016). However, scientific evidence is needed to support its supposed therapeutic efficacy.

Aims: To evaluate the effects of a tincture of leaves of *C. citratus* on vascular smooth muscle.

Methods: The effects on aortic contraction of anethanolic tincture of leaves of *C. citratus* at different concentrations were assessed in endothelium-denuded rat thoracic aortic rings, pre-contracted with 60 mmol/L of KCl.

Results: The tincture extract of leaves of *C. citratus* induced concentration-dependent relaxation in endothelium-denuded rat aortic rings with an IC₅₀ of 41.8 ± 5.2 mg/mL. The highest concentration studied (100 mg/mL) caused a 91.3 ± 3.4 % of vasodilation, while the lowest concentrations (0.03 to 10 mg/mL) hardly caused vasodilation. At 30 mg/ml a vasodilation of 28.8 ± 3.1 % occurred.

Discussion: These findings suggest that the relaxation effect of this tincture is endothelium-independent. Similar results were obtained with methanolic extracts of leaves (Runnie et al., 2004; Devi et al., 2012), stems, and roots of *C. citratus* and citral (Devi et al., 2012). The decoction of leaves of *C. citratus* showed some dose-related hypotensive effects given intravenously in rats and some weak diuretic effect when given orally in rats (Carbajal et al., 1989). All this assumes that the chemical components responsible for the vasodilator action are present in all the extracts. The results obtained with the *C. citratus* leaf tincture suggest that the plant may have some hypotensive potential.

Conclusions: The mode of action on the vasorelaxant effect caused by this tincture remains to be investigated. The findings from this study provide a scientific basis for the use of this plant in traditional medicine and merits further investigations.

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Phytochemical study and hypolipemiant effect of *Bidens odorata* Cav.

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Bidens odorata is a medicinal and edible plant known as "mozote blanco, aceitilla, acahual, mozoquelite" which is traditionally used in Mexico as a diuretic, hypoglycaemic, anti-inflammatory, antipyretic, antitussive, to treat gastrointestinal disorders, kidneys pain, and lung or respiratory diseases^{1,2}. This research describes the phytochemical analysis of *B. odorata* and the evaluation of the lipid-lowering activities shown by the ethanolic extract of the aerial part and one of the isolated metabolites, as well as their acute toxic effects.

Structural elucidation of novel metabolite was carried out using 1 and 2D NMR, infrared spectroscopy (IR) and mass spectrometry (ESI-MS). The evaluation of the hypolipidemic effect of the ethanolic extract and the glycosylated compound was tested in a murine model of hypercholesterolemia induced by diet and by Triton WR-1339. On the other hand, the LD₅₀ of the ethanolic extract was evaluated in mice ICR by the OECD protocol TG 423³.

Bidens odorata is a species widely distributed and used in the traditional Mexican medicine, however there are few reports in this plant. In the present study, it was demonstrated that its ethanolic extract showed lipid-lowering activity at doses of 100 and 1000 mg/Kg, while the new metabolite was active at doses of 50 and 100 mg/Kg. In addition, the LD₅₀ of the ethanolic extract was >2000 mg/Kg, meaning that this extract does not cause lethality or adverse effects, and no signs of organs alterations or tissue damage were observed⁴.

The ethanolic extract and isolated metabolite showed an important lipid-lowering effect, without lethality or secondary effect. The above results found in this study supports the documented traditional use of *B. odorata*.

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**Evaluation of the antifungal effect of the leaf extract of Sospó flower
(*Pseudobombax ellipticum*) for fungi: *Aspergillus flavus*, *Aspergillus niger*,
Aspergillus fumigatus and *Candida albicans***

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Introduction

Pseudobombax ellipticum is a tree with abundant flowering, it is a species native to Mexico, but it has been distributed throughout many tropical regions of the Americas as an ornamental and medicinal plant.

In different regions people have used the flower and leaf to alleviate respiratory diseases such as cough and asthma but there is still no study to prove it, however, because of the properties presented by the objective of this work is to evaluate the antifungal activity in four different strains of pathogenic fungi: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* using the young leaf extract of *Pseudobombax ellipticum*.

Method

Preparation of the extract

150 g are washed to remove foreign matter from the leaf and subjected to a drying at room temperature for 24 hours, then cut into small pieces and sample cartridges are made on filter paper to be loaded into the main chamber of the Soxhlet and passes the solvent (ethanol) running it for 7 cycles to obtain the desired material and finally concentrates by removing the solvent by means of a rotaevaporation.

Antimicrogram

It is used as PDA culture medium, once gelled the inoculation is made on each of the plates with the different test microorganisms.

Voriconazole is used as a positive control and ethanol as a negative control, then six dilutions are prepared, from 100 µg / µL to 600 µg / µL, at the end of each sensitisk it is immersed in the different tubes with the different concentrations and placed on the surface of each agar in triplicate. The plates are incubated inverted

at 36 ° C for 5-7 days and once the stipulated time has passed, the inhibition zones of each plate are measured.

Resultados/Discusión/Conclusión

When measuring the halos we can conclude that for *Aspergillus flavus* the concentration of the extract with the highest inhibition was 600 µg / µL, *Aspergillus niger* at 600 µg / µL, *Aspergillus fumigatus* at 200 µg / µL and *Candida albicans* at 600 µg / µL.

Although in all cases they showed an effect, the highest sensitivity was observed in the microorganisms *Aspergillus flavus* and *Candida albicans* with 4mm and 4.1 mm of halo.

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Evaluation of the antifungal activity of the extract of the *Acacia Cornigera* plant (ergot)

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Introduction

Over the years, humanity has evolved and with it, their lifestyle has adapted to the new needs by developing sciences and technologies, with the growth of this environment the microorganisms found in our environment have adapted and become more resistant, Among the most common are two families with different species of *Aspergillus* (*Flavus*, *Niger*, *Fumigatus*) and *Candida* (*Albicans*) which are fungi that seriously affect the respiratory system, have carcinogenic effects and are deadly for humans.

Currently the inhibition of fungal growth is controlled by chemicals which in routine consumption could be harmful, therefore natural solutions are sought with antifungal effects, *Acacia Cornigera* is a plant native to Veracruz that has medicinal properties with antidiarrheal functioning, its characteristic thorns (horns) have protein and detoxifying properties so the objective of this research is to verify the antifungal activity of the spines of this plant.

Method

The first step is the cultivation of the fungi *Aspergillus* (*Flavus*, *Niger*, *Fumigatus*) and *Candida* (*Albicans*) on plates with potato agar and dextrose (PDA) are incubated at 36 ° C for 24 hours and left for 4 days to observe the sporulation. With the extract of the spine of the *Acacia Cornigera* plant six dilutions are made with concentrations of 100, 200, 300, 400, 500 and 600µg mL⁻¹. With the 24h culture, five colonies were taken from each plant and suspended in 5ml of sterile water, where it is homogenized for 30s until its turbidity is observed.

Subsequently, the Kirby Bauer method was applied to determine the fungal inhibitory activity, using PDA agar plates, 5.41mm diameter filter paper sensidiscs and two controls, one positive (voriconazole) and one negative (ethanol), which helped us determine that the procedure was correct.

Finally, the inoculated plates were incubated at 35 ± 2 ° C for 24-30 and up to 48 hours in an inverted position.

Results/Discussion/Conclusion

Favorable results are obtained in the four areas, higher sensitivity is shown in *A. Falvus* with response in concentrations 100, 200, 300, 500 and 600 $\mu\text{L mL}^{-1}$ with inhibition zones of 3.99, 6.05, 4.32, 6.44 and 4.66 mm of radio and *A. Niger* in the concentrations of 100, 200, 300, 500 and 600 forming inhibitory haloes of 4.89, 6.16, 5.31, 4.07 and 4.64 mm radius. Greater resistance is shown in the fungi *C. Albicans* 200, 500 and 600 $\mu\text{L mL}^{-1}$ with halos of inhibition of 6.98, 5.75 and 5.71 mm of radius and in *A. Fumigatus* 300, 500 and 600 $\mu\text{L mL}^{-1}$ with haloes of 5.19, 5.95 and 5.04 mm in diameter. With this research, we conclude that the extract of the spine of the *Acacia Cornígera* plant is promising for the inhibition of fungal growth.

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Evaluación del D-005 mediante el ensayo de la morfología de la cabeza del espermatozoide en ratas

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Introducción

El D-005, sustancia lipídica del fruto de *Acrocomia crispera* (palma corajo), contiene una mezcla de ácidos grasos, principalmente láurico, oleico, mirístico y palmítico y ha mostrado efectos antiinflamatorios y antioxidantes en roedores. El objetivo de este trabajo fue determinar si el D-005 induce cambios en la frecuencia de distribución para las formas anormales en la morfología de la cabeza del espermatozoide y/o induce alteraciones en la concentración de espermatozoides.

Método

Se emplearon ratas Sprague Dawley machos, provenientes de un estudio de toxicidad subcrónica, distribuidos aleatoriamente en cuatro grupos experimentales de ocho animales cada uno: un control tratado solo con el vehículo Tween 65/H₂O y tres administrados con D-005 (200, 500 y 1000 mg/kg). A las 24 horas de la última administración, a cada animal se le realizó la necropsia y se extrajo el epidídimo derecho para obtener las muestras.

El conteo de espermatozoides se realizó en una cámara de Neubauer, se calculó la concentración de espermatozoides y los resultados se expresaron en 10⁶ células/mL. Para realizar el análisis de la morfología de la cabeza del espermatozoide, se siguió el criterio de clasificación basado en cabezas normales y anormales que incluye amorfas, banana, sin gancho y dos colas.

Resultados/Discusión/Conclusión

El número promedio de espermatozoides por cuadro y la concentración de espermatozoides de los animales tratados con D-005 fueron estadísticamente similares a los del grupo control. Los valores de espermatozoides obtenidos en los controles son similares a los de un estudio previo. Los promedios de espermatozoides normales, anormales totales y por diferentes formas anómalas en los grupos tratados fueron similares a los del grupo control, indicando la inocuidad genotóxica del D-005 sobre las células germinales masculinas. El esquema de tratamiento utilizado permitió estudiar las células que durante la diferenciación y el desarrollo estuvieron en contacto con la sustancia en estudio o sus metabolitos. Los resultados obtenidos en este ensayo demuestran que, a las dosis estudiadas el D-005 no interfiere en ninguna de las etapas del proceso de



diferenciación de las células germinales. Las ratas fueron tratadas por más de un ciclo espermático, por lo tanto no solo los espermatozoides analizados estuvieron bajo la acción de la sustancia de ensayo durante todo su ciclo celular, sino que los órganos sexuales y el sistema endocrino en su totalidad estuvieron sometidos a la acción de altas dosis de D-005, durante un período de tiempo lo suficientemente prolongado como para que se manifieste cualquier acción tóxica, que pudiera tener repercusión sobre la producción de espermatozoides. El tratamiento oral con D-005 (200, 500 y 1000 mg/kg) durante el período de espermatogénesis no redujo el número total de espermatozoides ni aumentó la frecuencia de formas anómalas, por lo cual dicha sustancia en estudio no presenta potencial genotóxico sobre las células germinales masculinas.

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Evaluación Genotóxica del D-005 en el Ensayo de Micronúcleos en Médula Ósea de Ratas

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Introducción

El D-005, sustancia lipídica del fruto de *Acrocomia crispata* (palma corajo), contiene una mezcla de ácidos grasos, principalmente láurico, oleico, mirístico y palmítico y ha mostrado efectos antiinflamatorios y antioxidantes en roedores. Este estudio evaluó el potencial genotóxico y citotóxico del D-005 utilizando el ensayo de micronúcleos en médula ósea de ratas.

Método

Se emplearon ratas Sprague Dawley, provenientes de un estudio de toxicidad subcrónica (90 días), las cuales se distribuyeron en cinco grupos experimentales de cinco animales por sexo: tres grupos tratados por vía oral con D-005 (200, 500 y 1000 mg/kg), un grupo control negativo que solo recibió volúmenes equivalentes del vehículo y un grupo control positivo (Ciclofosfamida-CF 20 mg/kg), administrado en dosis única por vía intraperitoneal, 48 horas antes del sacrificio. Concluido el período de administración a cada animal se le realizó la necropsia y se les extrajo el fémur para obtener las muestras. Se contabilizó la presencia de eritrocitos policromatófilos (EP) y normocromatófilos (EN) en 2000 células/animal. Además, se calculó la frecuencia de EP portadores de micronúcleos (MN-EP) en 2000 EP/animal.

Resultados/Discusión/Conclusión

No existieron diferencias significativas entre el grupo control negativo y los grupos administrados con D-005 en relación al índice EP/EN, indicador de la proliferación celular en médula ósea, lo que indica ausencia de efecto citotóxico del tratamiento a las dosis evaluadas; de modo que las células continuaron su fase normal de diferenciación, proliferación y maduración en la médula ósea. No se apreciaron diferencias entre los sexos en ninguno de los grupos incluidos en el estudio. Los índices EP/EN de los controles positivos CF (20 mg/kg) disminuyeron significativamente con respecto a los de los controles negativos, además se observó un incremento significativo de EP portadores de MN. La respuesta encontrada en el grupo tratado con CF para todos los indicadores, no sólo evidencia la acción citotóxica y clastogénica de este mutágeno referida por otros autores, sino que valida la sensibilidad del modelo empleado. La frecuencia de aparición de EP con MN, indicador de efecto genotóxico, tampoco mostró diferencias significativas entre los grupos tratados con

D-005 y el grupo control negativo y se mantuvo dentro del intervalo de frecuencias espontáneas reportadas para ratas de esta línea, así como la comparación entre los sexos no reveló la existencia de diferencias en ninguno de los indicadores estudiados. Los resultados obtenidos indican que el tratamiento oral con D-005 (200, 500 y 1000 mg/kg) no induce actividad clastogénica, ni citotóxica en médula ósea de ratas.

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Isolation and characterization of *Siparuna echinata* (Kunth) A. DC of the Province of Loja, Ecuador

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Introduction

Since ancient times, medicinal plants have played an important role in society [1], they have an inexhaustible source of chemical molecules with bioactive properties that start new treatments [2].

According to the Monitoring Center for the Conservation of the Environment, Ecuador is among the 17 megadiverse countries, which houses 10% of all plant species in the world [3] 30% of the entire Ecuadorian population belongs to different indigenous groups and therefore ethnobotanical knowledge is still used and transmitted to future generations [4]. As the research system progresses, natural products such as pure compounds or standardized plant extracts offer unlimited opportunities for new drug prospects due to the availability of chemical diversity [5]. The *Siparuna* genus of the Siparunaceae family comprise a diverse group of 235 plant species distributed in tropical regions of Central and South America[6]. This genus is characterized by biosynthesizing compounds such as sesquiterpenes [7], flavonoids [8] and isoquinoline alkaloids [10]; compounds among which importance has been given to biological activities, such as antibacterial, antimalarial, and antileishmaniasis[11].

Siparuna echinata (Kunth) A. DC. Commonly known as lemongrass or wild lemon. It's widely distributed in forest areas. Medical use: the leaves of this species are used as a poultice to relieve rheumatism and cure infections such as herpes or those generated by insect bites[12].

Method

The aerial parts of *Siparuna echinata* in flowering state were collected in the city of Loja and deposited in the Herbarium of the Private Technical University of Loja.

To obtain extracts from the leaves, the drying chamber was collected for three days at a temperature of 34 ° C. The previously ground material (730g) was subjected to the Soxhlet process for 12 hours with acetate solvents (EtOAc) and methanol (MeOH); by means of reduced pressure, the solvent is removed, obtaining the EtOAc 200g extracts in 27.4% yield and 87g methanol in 11.9% yield.



For the fractionation of compounds, we proceeded by means of chromatography on a CC column eluted in solvent mixtures according to the requirement of the metabolites later evidenced in TLC thin layer chromatography and identified by NMR Nuclear Magnetic Resonance.

Results

Table 1. Isolated metabolites *S. echinata*.

Fixed part	Compound
Ethyl acetate	Sesquiterpeno
	Flavone
Methanol	Flavonoid
Volatile part	
Essential oil	Terpenes
	Sesquiterpeno

Conclusiones

In the present project it has been possible to isolate sesquiterpene and flavone compounds in acetate; while in the flavonoid type methanol extract.

The quantification and characterization of the essential oil was obtained 28 compounds of monoterpene type as major and sesquiterpenes.

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Larvicidal indole alkaloids from *Tabernamontana cymosa* Jacq.

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Tabernaemontana cymosa is a species that is distributed in different countries of America, among which is Colombia; it is characterized by its leaves with tiny black spots on the underside and warty fruits (Abubakar and Loh, 2016). The biological activity of alkaloids has been widely studied and defensive functions in the plants have been attributed to them (Hamid et al., 2017; Danieli and Palmisano, 1986). Our research group has been studying the larvicidal activity of the indole alkaloids, isolated from the active fraction obtained by biodirected fractionation of the ethanol extract of the seeds of *Tabernaemontana cymosa*, and the possible mechanisms of their action on the larvae of *Aedes aegypti*, mosquito vector of viruses transmitters of tropical diseases such as, dengue, chikungunya and zika (Guzman and Kouri, 2004). In this paper we present the results that were obtained from the isolation and characterization of the indole alkaloids from the seeds of *T. cymosa* and their larvicidal activity (LC₅₀) against the *Aedes aegypti* mosquito in its larval stage. The extract of *Tabernaemontana cymosa* was prepared from 1000 g of seeds, dried and ground and subjected to cold maceration with 95% ethanol. Subsequently the extract was subjected to chromatographic fractionation by open column (5 cm x 60 cm), in normal phase with polarity gradient from Hexane to Methanol, and chemical verification of the presence of alkaloids using the Dragendorff reagent. The fraction soluble in chloroform showed abundance of alkaloids and a good larvicidal activity against larvae of *Aedes aegypti* mosquito in stages III (early) and IV (late) and from there, by different stages of isolation and chromatographic purification in open column and gradients of elution, six alkaloids of 21 previously identified by HPLC/MS were isolated in this active fraction; four of these alkaloids, namely Voacangine (1), 7-Hydroxyindolenin (2), 3-Oxo-Voacangine (3), Rupicolina (4), have previously been isolated from this plant and other species of the genus *Tabernaemontana* (Bao et al., 2013; Danieli and Palmisano, 1986), while that the other two alkaloids, 6-enyl-6-formyl-Voacangine (5) and 3-propan-2-one-Voacangine (6), are new compounds.

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Use of an internal membrane infusion of the gizzard of *Rhea pennata pennata* as a digestive

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Introduction

Ethnomedicine is a very prolific research field, especially in the Southern Patagonia of Argentina. This research work allows to develop a first scientific approach to a widely used remedy in rooms or posts as an alternative medicine: The “pesina”: This medicinal compound is the inner membrane of the muscular stomach of the *Rhea pennata pennata* (Patagonian choique) that after drying under natural conditions, it is finely powdered to prepare an infusion with it ⁽²⁾. The folk medicine of this Patagonian region uses “pesina” as a digestive mainly after heavy or abundant meals.

Method

The objective of this trial was to find "in vitro" the pharmacological properties of "pesina". The laboratory sought to recreate the possible action of the “pesina” and thus estimate the effects in vivo that are used as a curative. As biological material in the test, the internal membrane of the muscular stomach of *R. pennata pennata* provided by local inhabitants, totally pulverized, was used. Three trials were performed with their repetitions in order to estimate the activity of the “pesina”. 1 - Biuret reaction ⁽¹⁾ 2 - Action of the “pesina” in the coagulation of milk; 3 - Action of the “pesina” on the pH of a protein solution.

Results / Discussion / Conclusion

The results of the tests carried out were: In the first test, the following were: polypeptides, which are the specific reagents for the Biuret reaction. In the milk protein coagulation test, the precipitation of casein was determined visually as a film of milk forms on the walls of the test tubes. This is indicating the action of the “pesina” on the proteins. In the third test, a rapid decrease in pH was observed, decreasing from pH 6.5 to pH 4.9, not differentiating itself by the type of protein used. This is indicating the action of the “pesina” on the proteins. According to bibliographic sources the active component that has been isolated from the gizzard lining is called ventriculin. This substance was used in modern medicine at the beginning of the 20th century, at which time it was derived from pork stomachs. It had been prescribed primarily as a treatment of pernicious anemia, a condition that often resulted from malabsorption of vitamin B12, and for atrophic gastritis (also called chronic gastritis), one of the leading causes of pernicious anemia in adults. This

substance may have worked by two mechanisms: to provide an intrinsic missing factor secreted by the stomach lining that is necessary for the absorption of B12 and to stimulate the secretion of gastric acid and digestive enzymes, such as pepsin, that help release essential nutrients from food, B12⁽³⁾. It is concluded in this first approach to the subject that according to the chemical determinations developed in this study, the “pesina” contains Pepsin, a substance that acts to favor digestion through a proteolytic action and also stimulates the secretion of pepsinogen and hydrochloric acid. The study of the characteristics, properties and composition of ventriculin, possibly present in the “pesina”, opens up a very wide and necessary field of action to deepen.

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Evaluation of the antimicrobial efficiency of cornezuelo extract (*Acacia cornígera*)

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Introduction

The use of medicinal plants is a common practice in the world, because people prefer to naturally relieve their ailments, since some commercial antibiotics have developed resistance to pathogenic strains. An alternative is the use of ergot (*Acacia cornígera*) which is a tree that, according to traditional medicine, has properties to treat diseases such as infectious diseases. The following work presents an ergot study as a possible antimicrobial in pathogenic strains of *Escherichia coli* 11229, *Escherichia coli* 25922, *Escherichia coli* 10536 and *Pseudomonas aeruginosa*. (Soto, Pérez and Estrada, 2016).

Method

With the extract of the *Acacia cornígera* spines, dilutions were made at different concentrations, 200, 400, 600, 800 and 1000 $\mu\text{g mL}^{-1}$. To prepare the bacterial culture, the strains were inoculated and incubated for a period of 16 to 18 h. Subsequently, they were incubated for 3 h until they reached an absorbance in the spectrophotometer from 600 to 750 nm, equivalent to a bacterial suspension of 1.5×10^8 CFU / mL.

Finally, the antibiogram was performed on plates using the Kirby Bauer method, using sensidiscs, in addition to using a positive control (benzylpenicillin, amoxicillin with clavulanic acid and nitrofurantoin) and negative (ethanol) in each of the plates.

Results/Discussion/Conclusion

The processes that are carried out prior to the antibiogram, indispensable for carrying out the experiments, were successfully carried out. Regarding the evaluation of the antimicrobial activity of *Acacia cornígera* expressed through the antibiogram used by the Kirby Bauer method, it is concluded that: The four bacterial strains were inhibited with the extract of *Acacia cornígera*, showing greater antibacterial activity in the strain of *Pseudomonas aeruginosa*, since the inhibition halos formed by the concentration of 1000 $\mu\text{g mL}^{-1}$ showed greater diameter with measures of 4.2 and 4.4 mm compared to the measurements of the halos formed by the antibiotic that showed a measurement of 3.4 mm for both essays.



The strain that showed the greatest resistance in this investigation was that of *E. coli* 25922, since the average of the highest inhibition halo obtained in the different concentrations was obtained by concentration 2 with a measurement of 2.3 mm, which is well below to the measurement obtained by the inhibition halo formed by the antibiotic, which was 3.6 mm.

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Sustainable nutrition and sustainable global society

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Abstract

The research, Sustainable Nutrition and Sustainable Global Society, was carried out in the population of St. Louis, the sample was applied in the Marie Pierre Curie Scientific Club of the I.E. San Juan Macías, 2015.

The objective was to reduce malnutrition and improve student health, through a learning program that lasted 2 years, making known and consuming healthy and nutritious foods. The scientific quasi-experimental method was used and the design was correlational research for the variables: Sustainable Nutrition and Sustainable Society. The results achieved are presented in tables 1, 2, 3, which shows that the greatest importance of sustainable nutrition is greater sustainable global society.

It was confirmed that sustainable nutrition in students does influence the healthy sustainable society.

Key words: Sustainable nutrition, Sustainable society



The *n*-butanol extract obtained from the inner bark of *Tabebuia rosea* (Bertol) DC in combination with apicidin induces apoptosis in leukemia cells

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Introduction

Natural products are an important therapeutic strategy to arrest the proliferation of cancer cells. It is necessary to explore the potential of extracts obtained from *Tabebuia rosea* (Bertol) DC since, in this species, molecules such as quinones, iridoids, flavonoids, and several molecules with anti-inflammatory, anti-oxidant, and anti-proliferative activity have been reported. Therefore, this work aimed to determine the effect of the *n*-butanol extract from *Tabebuia rosea* (Bertol) DC in combination with the Histone Deacetylase Inhibitor (HDACi) apicidin in leukemia cells.

Method

The cytotoxic effect of the extract was evaluated at 10-400 µg/mL during 12, 24 and 48 h, using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric method on THP-1, Jurkat and Peripheral Blood Mononuclear Cells (PBMC). Apoptosis, mitochondrial membrane potential, and cell cycle effects were evaluated by flow cytometry with annexin v/7-AAD, JC-1, and PI. p38 and Bcl-2 were detected using the western blot method. Cellular morphology and nuclear fragmentation were detected by fluorescence microscopy of cells stained with Hoechst 33342 and Propidium Iodide (PI), respectively.

Results/Discussion/Conclusion

The IC₅₀ of the extract at 12, 24, and 48 h of exposure was 35.3, 44.7, and 36.4 µg/mL, respectively. The increase in the percentage of cells in early apoptosis is directly proportional to the extract concentration. However, only significant differences were observed between the low (25 µg/mL) and high (100 µg/mL) concentrations, with apoptosis percentages of 15.62% and 20.62%, respectively (p<0.05). Concerning the mitochondrial membrane potential, all the concentrations used did increase the depolarization of the membrane in more than 80% of the cells, when pre-treatment with apicidin was carried out. The combination

of apicidin and the extract significantly increased the percentage of cells in the G0/G1 phase concerning the control ($p > 0.001$). The p38 protein was phosphorylated after treatment with the extract at 50 and 100 $\mu\text{g/mL}$. However, the combination with apicidin did not affect p38 phosphorylation. The levels of the Bcl-2 protein decreased in the cells treated with the extract and apicidin concerning the control. Fluorescence microscopy showed an increase in the number of apoptotic cells in the cells treated with the extract and the combination with apicidin. The combination of the *n*-butanol extract and apicidin induced cytotoxic and apoptotic effects on THP-1 cells, through the intrinsic pathway (a decrease in the mitochondrial membrane potential and Bcl-2 production). This combination caused G0/G1 cell cycle arrest and effect on the phosphorylation of p38.

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Molecular diversity and biological activities of kaurane diterpenes

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Diterpenes represent a vast class of natural isoprenoid, they are classified in acyclic (phytanes), bicyclic (labdanes, clerodanes), tricyclic (pimaranes, abietanes, cassanes, rosanes, vouacapanes, podocarpanes), tetracyclic (trachylobanes, kauranes, aphidicolanes, stemodanes, stemaranes, beyeranes, atisanes, gibberellanes), macrocyclic diterpenes (taxanes, cembranes, daphnanes, tiglianes, ingenanes) and mixed compounds, in accordance with the number and the cyclization patterns displayed by their skeleton [1]. They are found mainly in plants and fungi, although diterpenes have also been found in marine organisms and insects as well [1]. Most diterpenes are specialized metabolites and may have roles in ecological interactions of plants and contribute to plant fitness. These compounds showed a wide range of biological activities such as antimalarial, anti-inflammatory, antidiabetic, antibacterial, cytotoxic, antitumoral, insecticidal, and several others activity². Moreover, diterpenes show a wide range of targets (heat shock proteins, growth factors, transcription factors, enzymes) and qualify as privileged structure for biological and biomedical research. [2,3]. In the last few years our research group was involved in a research project aimed to the search of diterpenes with significant biological functions and/or unknown chemical structures from a variety of different plants [4,5].

Owing to our interest in the field of plant diterpenes, we have developed approaches to diterpene target or lead(s) identification based on chemical genetic procedures, supported by spectroscopic, and spectrometric data and biochemical investigations.[2]The most promising diterpenes then underwent an evaluation of the target(s) modulatory activity by means of a panel of chemical and biological approaches, including Saturation-Transfer Difference -NMR, Surface Plasmon Resonance measurements, biochemical and cellular assays, limited proteolysis, and molecular docking. Our strategies and studies in the described research area, are demonstrated by several results from recent and ongoing research projects [4,5].

Keywords: Diterpenes, SPR, MS, biological activity

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Pefil lipídico de la ancestral semilla de maní del inca extraída por proceso no térmico del cantón San Vicente, Manabí, Ecuador

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Introducción

Sacha inchi (SI) (*Plukenetia volubilis* Linnaeus), conocido también como, maní silvestre, maní del Inca, maní sacha o montaña maní, es una planta oleaginosa que pertenece a la familia de las Euphorbiaceae. En un principio y hasta la actualidad ha sido cultivado en las tierras bajas de la amazonía peruana, y siendo plantado durante siglos por la población indígena, ha sido un componente de la dieta de varios grupos nativos de la región (Gutiérrez et al., 2011)(Chirinos et al., 2013). Según (Muangrat et al., 2018) el porcentaje de obtención de este aceite prensado a 60°C es de 37.97%, el cual tiene un porcentaje aproximado de 92% de ácidos grasos poliinsaturados (AGP) como ácido alfa linolénico (18:3n-3, ácido α -linolénico) y ácido linoleico (18:2n-6, ácido linoleico), (Fanali et al., 2011)(Cisneros et al., 2014). Este tipo de ácidos grasos presenta una o varias ligaduras entre sus carbonos, y dependiendo de su ubicación se los denomina α -3, 6 o 9 (Badui Dergal and Pedroza Islas, 2013). Según (Araújo-Dairiki et al., 2018) indica que estos ácidos grasos tienen efectos beneficiosos que incluyen la capacidad de disminuir los niveles de glicéridos, prevenir los trastornos cardiovasculares y acción antitrombótica, además ciertos experimentos han demostrado que este aceite tiene una alta capacidad antioxidante, ayudando a reducir el daño al ADN debido a la oxidación (Takeyama and Fukushima, 2013). Últimamente el interés por estos nutrientes se ha visto imputada gracias a un conjunto de publicaciones, demostrándose así que la ingesta de grasa depende de la calidad de esta, es decir del tipo de ácido graso predominante(Carrillo Fernández et al., 2011). Convirtiéndose en un reto el estudio de SI, tanto como material oleaginosa y también por las funcionalidades biológicas que podrían derivar de su aceite y/o de sus extractos(Castaño T et al., 2012).

Por otra parte, el consumo de aceite de oliva (AO) se ha visto incrementado debido a los beneficios de los aceites vegetales ya que la composición del AO, el cual tiene en su interior una gran cantidad de ácidos

grasos monoinsaturados, en particular ácido oleico. Además, de estar presentes el ácido α -linolénico (α -3) y ácido linoleico (α -6) los cuales el cuerpo humano requiere y no puede sintetizar (Piscopo et al., 2016).

Actualmente, el aceite de pescado en capsulas es comercializado globalmente debido a que es rico en ácidos grasos poliinsaturados α -3 [40.91% según(Paucar-Menacho et al., 2015)], ácido eicosapentaenoico (EPA) (20: 5, n-3) y ácido docosaheptaenoico (DHA) (22: 6, n-3)(van der Tempel et al., 1990). Sin embargo, la contaminación ambiental ha provocado la acumulación de metales pesados y dioxinas en los peces, y debido a esto, se cuestionan los beneficios de obtener ácidos grasos insaturados a partir de peces. (Maurer et al., 2012)

Personas que no pueden comer pescado diariamente o lo hacen con poca frecuencia, pueden complementar su ingesta de ácidos grasos α -3 con aceites vegetales (Strobel et al., 2012). Sin embargo, como estos ácidos grasos se oxidan fácilmente a altas temperaturas, sus aplicaciones potenciales son limitadas. Como tal, el análisis de nuevas fuentes de ácidos grasos α -3, sería extremadamente beneficioso desde el punto de vista de la salud humana. (Takeyama and Fukushima, 2013).

El objetivo de este estudio es extraer el aceite de SI por un prensado en frío y realizar una caracterización de este, para luego poder realizar una comparación entre los aceites ricos en ácidos grasos insaturados, como son el aceite de pescado y el aceite de oliva.

Phytomodulatory hydrogel based in laticifers proteins from *Calotropis procera* and plant hemicelluloses as an alternative natural product for wound healing

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Introduction

Calotropis procera (Ait.) R. Br. is well known for being a laticifers plant and for its medicinal properties. Its latex has already been used in traditional medicine in the treatment of dermis disorders. Scientific studies have shown that laticifers proteins isolated from *C. procera* have several pharmacological properties and their anti-inflammatory, antiseptic, analgesic and wound healing effects are reported. The aim of this work was to evaluate the effect of the CpPII protein fraction of *C. procera* on cicatricial process modulation in experimental wound models.

Method

A hydrogel (Hg) was developed using natural hemicelluloses (galactomannans) from *Caesalpinia pulcherrima* (Cerqueira et al., 2009), as excipient, and laticifers proteins (CpPII), rich in proteolytic enzymes as active principle. In the present study, laticifers proteins containing proteolytic activity (CpPII) or inhibited with iodoacetamide (IAA) were incorporated at different concentrations (w/v) in galactomannan hydrogels (Hemi) to obtain the following formulations tested: HgCpPII 0.2%, HgCpPII 0.5% and HgCpPII-IAA 0.2%.

Male Swiss mice were used for in vivo tests (wound healing and dermal irritability) according to experimental procedures approved by the institutional ethics committee under number 64/2014. Tissue modifications and target mediators of cicatricial response were evaluated in excisional wound models (1cm²).

Results/Discussion/Conclusion

Wounds treated with the CpPII fraction showed modulation of inflammatory mediators and reduction of lipid peroxidation (MDA). There was intense stimulation of the inflammatory and proliferative phase of healing process. Furthermore, fibroblasts activation and collagen deposition resulted in a significant increase in the rate of wound contraction as well as tissue re-epithelialization when compared to control groups ($p < 0.05$). Wound healing assays with the CpPII fraction inhibited by iodoacetamide (20mM) showed that the latex pharmacological properties were not only associated with the presence of proteolytic activity proteins. The HgCpPII hydrogel did not induce dermal irritation and efficiently stimulated wound healing by acting on the different phases of the healing process. Hemicelluloses, initially proposed as a delivery vehicle for immunomodulatory proteins, were involved, at least in part, in modulating the process possibly acting as antioxidants. This study suggests a new application for plant hemicelluloses as vehicles using *C. procera* latex immunomodulatory proteins as useful substances in the treatment of wounds. However, further investigations are required for the proposed formulation to be tested in other wound healing models (Vasconcelos et al., 2018).

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Antidiabetic effects of *Annona diversifolia* Safford (Annonaceae): α -glucosidase inhibition, SGLT cotransporter inhibition and insulin secretion

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Introduction

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels resulting from insulin resistance or inadequate insulin secretion (Perez-Diaz, 2016). In the world, DM is one of the most frequently non-contagious disease that affects more than 371 million people and in 2012 caused the death of 1.5 million of people (ADA, 2016). The treatment of the DM frequently implicates use of SGLT cotransporter inhibitors, α -glucosidase inhibitors, and sulfonylurea agents. The control of the DM in Mexico also practiced by population through the use of medicinal plants, such as *Annona muricata*, *A. cherimola*, *A. squamosa*, *A. glabra* and *A. diversifolia*, which have been evaluated experimentally, demonstrating antihyperglycemic activity (Andrade-Cetto *et al.*, 2005; Calzada *et al.*, 2017; Arrollo *et al.*, 2009; Esquivel-Guitierrez *et al.*, 2012). However, the mechanism of this action have been poorly explored. The aim of the present work was to evaluate the potential antihyperglycemic of the ethanol extract of the leaves from *A. diversifolia* (EEAd) on three different action pathways: first, like an α -glucosidase enzyme inhibitor, second, like a SGLT cotransporter inhibitor and finally as a secretagogue.

Method

The α -glucosidase enzyme inhibitor, was measured by oral sucrose and lactose tolerance test (OSTT and OLTT), using acarbose as a pharmacological control. Second the SGLT cotransporter inhibition by oral glucose tolerance test (OGTT), using canagliflozin as a pharmacological control. These test were performed in fast normoglycemic Balb/c strain mice (FNM, 107.7 ± 3.6 mg/dL of glucose), the extract were evaluated at 200 mg/kg, acarbose and canagliflozin were evaluated at 50 mg/kg, they were administrated intragastric via using an esophageal cannula, 30 minutes after the products administration the carbohydrates were given (1.5 g/kg for sucrose and lactose and 3g/kg for glucose). In both cases after the administration of the carbohydrates the blood glucose levels was measured at 2 and 4h. The last mechanism of action was insulin secretion, this was evaluated on pancreatic cells isolated from Sprague Dawley rats; the cells were stimulated with alone *A. diversifolia* extract (400 μ g of extract/mL), alone glibenclamide 1600 μ M (Gb block

the K⁺ATP channels and promotes insulin secretion), alone diazoxide 4000 μ M (Dz, opens the K⁺ATP channels and inhibits the insulin secretion), 4000 μ M Dz with *A. diversifolia* (400 μ g of extract/mL), and 4000 μ M Dz with glibenclamide (1600 μ M). Insulin levels were measured with an immunochromatographic test kit from VEDALAB®.

Results/Discussion

The results showed that in OSTT and OSTT, the EEAd significantly decreased blood glucose levels in FNM at 2 h after lactose or sucrose load like acarbosa, an α -glucosidase inhibitor. In the case of OGTT, it was observed similar effect in FNM mice at 2 h after a glucose load like canagliflozin, a sodium glucose transporter 2 (SGLT2) inhibitor. On the other hand, EEAd increased significantly the insulin secretion as well as the pharmacological control (glibenclamide).

Conclusion

The EEAd possesses *in vivo* antidiabetic effect, exerting such effect through different action pathways that involve antihyperglycemic (α -glucosidase and SGLT cotransporter inhibitor) and hypoglycemic (insulin secretion) actions. These results explain in part the antihyperglycemic activity demonstrated by *A. diversifolia* and the use of these species on the Mexican folk medicine for the treatment of DM.

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Characterization of oleoresin extracted from tomato waste using carbon dioxide in sub and supercritical conditions

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Introduction

The food industry is characterized by a high number of wastes, nowadays they are used for agriculture or animal feed; in particular, in Italy, the tomato processing industry produces around 150 thousand tons of wastes each year (2-6% of the raw material). Tomato waste is characterized by high amounts of bioactive compounds such as carotenoids, polyphenols, vitamin E and essential fatty acids. In this work oil extracts from skins and tomato waste were produced using carbon dioxide in sub and supercritical conditions. Compositions of the oils were analyzed and compared to the composition of oil obtained by means of solvent.

Method

Skins and tomato waste were dried at 60°C and ground to get particle size of 1 mm. Skins and tomato waste were separately submitted to extraction by supercritical carbon dioxide, liquid carbon dioxide and hexane-dichloromethane mixture solvent. Supercritical and liquid CO₂ were carried out also using ethanol 10%. Carotenoids and tocopherols were determined in the extracts by means of high-performance liquid chromatography and spectrophotometry. Total polyphenols were determined via HPLC Diode Array/MS detector and using Folin-Ciocalteu method. The antioxidant capacity was measured via the DPPH method.

Results/Discussion/Conclusion

The best extraction parameters with subcritical CO₂ were pressure 150 bar, temperature 20°C and flow 5 ml/min, while for the extraction with supercritical carbon dioxide parameters were 340 bar, 60°C and 10 ml / min. Extraction from waste with CO₂ supercritical conditions with ethanol provided the highest yields in oleoresin (12.7-12.9%), while the extracts obtained from skins with supercritical carbon dioxide without ethanol had the highest quantities of lycopene (205.7 mg/100g oil), β-carotene (75.1 mg/100g oil), a and

γtocopherols (800.2 and 5756.0 mg/kg oil respectively). The highest quantity of polyphenols (93 mg GAE/g oil) was found in oil from skin by supercritical carbon dioxide with ethanol.

The oleoresin extracts from skins and tomato waste using carbon dioxide in sub and supercritical conditions showed a great value in its antioxidant capacity (+780%) due to the presence of carotenoids, polyphenols, and tocopherols compared to the extracts obtained with solvent.

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The local utilization profile of *sclerocarya birrea* (marula) in muswodi, limpopo province, south Africa

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Introduction

Understanding the uses of indigenous plants that are of economic importance to local communities is very much important in rural development strategies. *Sclerocarya birrea* commonly known as 'marula', is an indigenous fruit tree species that is widely used in South Africa. It belongs to Anacardiaceae family. The purpose of the study was to unravel the impact of the utilization of *Sclerocarya birrea* species on its population.

Method

Ethnobotanical utilization of the species was collected from thirty informants (23 females and 7 males) using semi-structured interviews. The informants were selected through convenient sampling approach where people who were available and willing to be interviewed were interviewed after obtaining prior informed consent.

Results/Discussion/Conclusion

The fruits are the most utilized part (63%) followed by the barks (24%) and the leaves (10%). Most of the informants indicated that they utilize the fruits in juice (30%) and wine (30%) making. The species was also reported to be used in traditional medicine (6%). This study clearly brings to light the deepened utilization of *Sclerocarya birrea*. It suggests that a management plan be put in place in communal areas to monitor the harvesting of *Sclerocarya birrea* since it is a protected species in South Africa.

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Componentes de la diversidad biológica empleados por las familias manabitas en la medicina natural y tradicional

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Introducción

La flora constituye el principal componente de la diversidad biológica empleado por las familias ecuatorianas para uso medicinal, también se han registrado especies de la fauna y hongos, pero en menor cuantía. Se estima en promedio, que la población utiliza más especies de la flora, que de fauna y hongos para uso medicinal y -en general- se han realizado investigaciones para rescatar los conocimientos ecológicos tradicionales de dichas especies y la importancia de éstas sobre los grupos humanos. Se conoce que las enfermedades crónicas constituyen la primera causa de morbilidad y también de mortalidad en Ecuador, la vigilancia de ellas constituye un elemento importante para alertar al sistema de salud sobre su evolución. Así, se ha considerado trascendente continuar dichos estudios y actualizar los principales componentes de la biodiversidad empleados por las familias montubias en la medicina natural y tradicional en las áreas protegidas de mayor interés económico conservacionista de la diversidad biológica como son el Parque Nacional Machalilla, entre otras con el propósito de analizar el papel que en la vida de las familias desempeñan, priorizando las enfermedades crónicas.

Son pocos los estudios sobre los componentes de la biodiversidad utilizados en la medicina natural y tradicional por la población montubia. Con el trabajo se cubrió este vacío de información para las zonas rurales de Membrillar, Pedro Pablo Gómez y Julcui, Manabí, y está relacionado con las especies de la flora y la fauna que son utilizadas por las familias ecuatorianas. La dispersión y desactualización de la información que posee la familia ecuatoriana sobre el conocimiento de la flora y la fauna medicinal, presentes en los ecosistemas forestales, priorizando las especies usadas para tratar los principales problemas de salud, enfatizando en las enfermedades crónicas que constituyen la primera causa de morbilidad y mortalidad en Ecuador. Se promovió el desarrollo participativo mediante el desarrollo de mini-industrias para el procesamiento de plantas medicinales.

Método



El estudio constituyó un inicio de las investigaciones desarrolladas en el marco de la investigación de la Universidad Estatal del Sur de Manabí (UNESUM), vinculado a los objetivos de trabajo de la Carrera de ingeniería Forestal. Se enmarca dentro del Programa Ecoturístico-Forestal.

En esta ocasión, se realizó la investigación con un enfoque etnobiológico y ecológico teniendo en cuenta la falta de estudios que comparen los componentes de la biodiversidad más utilizados por los residentes rurales, la investigación empírica incluyó entrevistas, encuestas y talleres participativos. Se identificaron los componentes de la flora y la fauna que están siendo más explotados por los residentes.

Parámetros de investigación: Las preguntas de investigación de acuerdo a los objetivos fueron:

- 1- ¿Cuáles son los componentes de la biodiversidad utilizados por las familias ecuatorianas en la medicina natural y tradicional?
- 2- ¿De dónde se extraen estos componentes (bosque natural, antropizado, jardines, etc)?
- 3- ¿Qué enfermedades curan y cómo es su preparación?

El estudio planteado contribuyó, entre otros aspectos, como base para futuras investigaciones de bioprospección, así como para la documentación de la información con respecto a los aspectos ecológicos y usos medicinales de las especies utilizadas por las familias ecuatorianas. Permitió la identificación de factores (edad, género y nivel educacional) que influyen en el patrón de uso de esos conocimientos en Manabí, y sus implicaciones en el desarrollo de las familias que viven cerca de las áreas boscosas. Proporcionando información útil para la industria farmacéutica con vistas a identificar nuevas fuentes naturales de interés medicinal, así como para los profesionales de la salud en particular, sobre cómo usar de modo más provechoso la relación de los humanos con los principales componentes de la biodiversidad empleado en la medicina natural y tradicional. La información obtenida se podrá contrastar con los datos sobre usos medicinales de la biodiversidad encontrados en otros países.

Resultados/Discusión/Conclusión

Por primera vez, se realizó una investigación etnobiológica, teniendo en cuenta las especies utilizadas por las familias manabitas para la medicina natural y tradicional con énfasis en las empleadas para la cura de las enfermedades crónicas.

En el ámbito social, el estudio permitió identificar las afecciones que más padecen las familias manabitas y las formas de cura mediante la medicina natural y tradicional, lo que permitió brindar las medidas de conservación para la biodiversidad sobreexplotada con el objetivo de que no se pierdan los principales componentes de la flora y la fauna utilizados. Los resultados están siendo divulgados en revistas y eventos científicos, los cuales permiten la socialización de las investigaciones científicas desde la Universidad Estatal del Sur de Manabí.

Entre los impactos potenciales en el ámbito económico, la investigación, no tiene impacto potencial desde el punto de vista económico, pero el valor metodológico de este estudio se sustenta principalmente sobre su potencial como herramienta de referencia para mejorar el proceso de conservación de la diversidad biológica.

Com estos resultados se espera responder a la Política Nacional Forestal, así como al Programa de Desarrollo del País, en sus tres ejes fundamentales.

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Traceability of Mediterranean dried figs using mineral composition

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Introduction

The common fig (*Ficus carica* L.), belong to botanical family Moracea, is widely grown in the world for its edible fruit, sweet and succulent. The minerals content of thirty-six dried figs samples collected from three Mediterranean areas in 2018 was presented in this study. The aim of this research was to evaluated if the mineral elements amounts are significantly different among the production regions. Samples grown in Italy (n=20), Greece (n=8) and Turkey (n=8) were involved in this study.

Method

Approximately 25 g of dried figs were cut into small pieces and were milled with a teflon mortar to avoid metal contamination. About 0.5 g of each sample were accurately weighed into acid-pretreated PTFE vessels, added with 1mL of Re internal standard, and then were digested with 7 mL of 65% HNO₃ and 1mL of 30% H₂O₂ using a closed-vessel microwave digestion system. After microwave digestion, mineral concentrations were determined by ICP-MS. Method validation was carried out according to EURACHEM criteria. All samples were analyzed in batches, with blank samples and known standards. All analyses were carried out in triplicate. Statistical methods were conducted on one starting multivariate matrix constituted by 36 cases (dried figs samples under analysis) and 12 variables (concentrations of minerals determined in analyzed samples). The data were subdivided into three groups according to the production area. The first step concerned the study of significant differences, among the samples obtained from the three production areas, by Kruskal-Wallis test. Then, the data sets were normalized and lastly Factor Analysis with Principal Components extraction was performed to relate mineral concentrations in dried figs to the production area.

Results/Discussion/Conclusion

The samples were found to be a good source of potassium (average content between 3.50±0.20 g/kg and 8.12±0.88 g/kg), calcium (between 1.46±0.24 g/kg and 4.49±0.28 g/kg), magnesium (between 0.84±0.04 g/kg and 1.28±0.15 g/kg), and sodium (between 0.45±0.04 g/kg and 0.70±0.11g/kg). Considering cadmium and lead, the concentrations were always below the regulated values (0.2 mg/kg and 0.4 mg/kg, respectively) and below the quantification limit in all Italian samples, while chromium was detected in all samples from Greece (0.05±0.01 mg/kg) and Turkey (0.03±0.01 mg/kg), and in 65 % of samples from Italy (0.08±0.02mg/kg). Thus, our results are confirmed the good figs quality related to their essential trace

elements content and are highlight the safety of these food products correlated to intake of toxic elements. Furthermore, statistical analysis of the results pointed out that the mineral elements amount is significantly different among the three sample groups, and so it is possible to discriminate figs according their geographical origin by PCA.

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Preliminary phytochemical study of the leaves of yawar panga (*Aristolochia cf. cauliflora* Ule.)

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Introduction

Peru is one of the twelve countries with the greatest biological diversity in the world, known as megadiverse countries, both for the number of species and genetic resources and for the variety of ecosystems. It is estimated that it has 25 thousand known plant species, of which 5354 are endemic to our country¹.

This plant richness allowed the development of traditional media and the use of medicinal plants in the medical practice of the different pre-Hispanic cultures of our country, in its three regions, Costa, Sierra and Amazonia, traditional use that continues to this day.

The leaves of Yawar Panga (*Aristolochia cf. cauliflora* Ule.) Are used in the Peruvian Amazon for presenting emetic properties and in the treatment of bronchial conditions². This plant was collected in the Botanical Garden of the Takiwasi Center (Tarapoto, San Martin region, Peruvian Amazon), where they use the decoction of the leaves in the treatment of their patients.

In the literature no phytochemical studies have been found about this plant.

Methodology

After performing the qualitative analysis of the Yawar Panga leaves (*Aristolochia cf. cauliflora* Ule.), The study of the apolar extract was carried out, macerating 501 g of leaves in petroleum ether, obtaining 4.5 g of Organic Raw Extract (EBO-ether).

This extract, EBO-ether, was fractionated on a chromatographic column (CC: ether, ethyl acetate, chloroform and methanol), obtaining 25 fractions, grouped into 6 groups (G1 to G6) of which, G4 evidenced the presence of sterols by thin layer chromatography (TLC).

The G4 was fractionated by CC (ether, ethyl acetate, methanol) into 83 fractions, which were grouped into 9 groups (G1' to G9'), which when analyzed by CCF, G4' evidenced the presence of sterols. Therefore, this fraction was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS).

Results and conclusions



The GC-MS analysis of the apolar extract (G4') of the leaves of Yawar Panga (*Aristolochia cf. cauliflora* Ule.) Indicates the presence of three sterols: campesterol (7.81%), stigmasterol (7.63%) and, mainly, γ -sitosterol (30.31%).

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Flavanones and flavones from leaves of *Chromolaena tacotana* (Klatt) R.M. King & H. Rob. and determination of its antioxidant activity

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Introduction

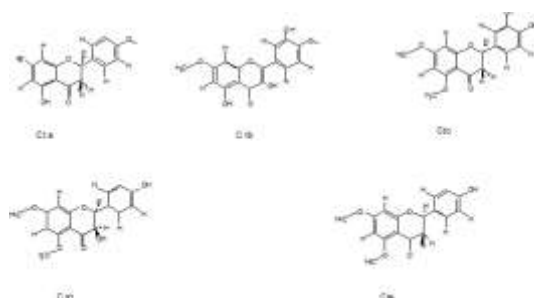
Chromolaena tacotana commonly called sanalotodo for its medicinal properties not scientifically proven, is a species that is recognized as a producer of flavonoids. In a first investigation in leaf extracts were found flavonoids: 3,5,4'-trihydroxy -7- methoxy flavone, 3,5,8- trihydroxy -7,4 '-dimethoxy flavone, 5,7 were identified, 3', 4' - tetrahydroxy- 3-methoxyflavone and 5,4'- dihydroxy -7- methoxyflavanonol that showed important antioxidant and cytotoxic activity (Rodriguez, 2018), so we continued with the identification of their other flavonoids whose results are presented in this publication. This type of compounds are recognized as active ingredients in multiple plant sources, both food and medicinal [Tepe et al, 2007],[Veeramuthu 2017]and their total content in extracts and fractions of leaves and inflorescences of plants must be known to understand the mechanism by which they lead to out your action. [Phan, 2001],[Santander,2007],[Torrenegra, 2016],

Methods

The leaves (794 g) were extracted in soxhlet with CH₂Cl₂, the extract (134 g) was flocculated with EtOH: water 1: 1 to remove fats and chlorophylls, the aqueous portion was extracted 1/1 with CH₂Cl₂ which was concentrated in vacuo to obtain the flavonoid block. 20 g of extract were separated by column chromatography with Si gel (40-60 μm) and RP18 (20-40 μm), the flavonoids were isolated using as eluents CHCl₃ and mixtures of CHCl₃ and MeOH and MeOH: water and purified with hexane and MeOH according to the solubility of each flavonoid. Identification was carried by means of U.V spectra with displacement reagents (AcONa, MeONa and H₃BO₃) and mono and two-dimensional ¹H NMR and ¹³C NMR spectra. The evaluation of the antioxidant activity was carried out by means of the techniques DPPH• and ABTS•⁺ [Huang 2005],[Suja F 2004],[Brand 1995]. For the UV-Vis spectra, solutions of 10mg / L in MeOH were prepared for each flavonoid, by spectral scanning between 230 and 500 nm in the Jenway 6504.31 spectrophotometer, reagents were added (MeONa, AcONa, H₃BO₃, AlCl₃ and HCl) in order to know information about the presence of OH-, OCH₃ groups in the molecular structure. The ¹H NMR and ¹³C NMR spectra were taken on a Bruker 300 MHz nuclear magnetic resonance machine.

Results/Discussion/Conclusion

At this stage of the investigation, five flavonoids were obtained: (Cta) 5,7 dihydroxy-4'-methoxy flavanone, (Ctb) 5,4'-dihydroxy-7,4'-dimethoxyflavonol; (Ctc) 3',4'-dihydroxy-5,7-dimethoxy-flavanone; (Ctd), 3,4'-dihydroxy -5,7- dimethoxyflavanone; (Cte) 4'-hydroxy-5,7-dimethoxyflavanone; The IC₅₀ of antioxidant activity for the flavonoids were 6,27µg / mL for Ctb and 53,31 µg / mL for Ctd, another flavonoids , Cta,Ctc Cte showed low activity, positive control quercetin showed an activity equivalent to an IC₅₀ of 8,67 µg / mL, the flavanones presented the lowest inhibition of free radicals as expected. In conclusion this plant is a good source of flavonoids with potencial medicinal activity.



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Optimization of hydrolysis conditions for the production of an antioxidant whey protein hydrolysate

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Introduction

Whey proteins are an important source of biologically active peptides that can be released by enzymatic hydrolysis. This process can be affected by several factors such as the enzyme specificity; the nature, the concentration and the extent of denaturation of the protein used as a substrate, and the physicochemical conditions applied during the hydrolytic process. For this reason, the objective of this study was to optimize the enzymatic hydrolysis of a whey protein concentrate to obtain a hydrolysate with antioxidant potential.

Method

A protein concentrate obtained by ultrafiltration of whey was hydrolyzed with alcalase (45°C, pH 8.0). The hydrolysis conditions, enzyme-substrate ratio (1:20 - 1:230, w/w) and time (1-9 h), were optimized using the response surface methodology with central composite design (16 assays). The soluble protein content, free amino acids, degree of hydrolysis and antioxidant activity (ABTS radical scavenging activity, determination of reducing power and iron-chelating activity) were determined.

Results / Discussion / Conclusion

The soluble protein content, free amino acids and degree of hydrolysis varied between 6.83 - 52.95 mg.g⁻¹, 10.75 - 27.22 mg.g⁻¹ and 22.15 - 36.22%; respectively. The antioxidant activity by the ABTS method fluctuated between 214.41 – 255.31 μmol trolox.g⁻¹. Additionally, the hydrolysates showed a reducing power between 3.42 - 5.15 μmol trolox.g⁻¹ and an iron-chelating capacity between 52.45 - 74.20%. The enzyme-substrate ratio and time significantly affected the soluble protein content, free amino acids, degree of hydrolysis and reducing power. A decrease in the concentration of soluble protein and an increase in the free amino acid content, degree of hydrolysis and reducing power were observed when both parameters increased. The most adequate conditions to obtain a hydrolysate with antioxidant potential were: enzyme-substrate ratio, 1:20, and time, 7 h. The hydrolysate obtained under optimal conditions presented 6.79 ± 0.55 mg.g⁻¹ of soluble protein, 26.02 ± 1.63 mg.g⁻¹ of free amino acids and a degree of hydrolysis of 35.67 ± 0.67%. The antioxidant activity by the ABTS model was 215.57 ± 9.63 μmol trolox.g⁻¹, the reducing power

of $4.41 \pm 0.04 \mu\text{mol trolox.g}^{-1}$ and an iron-chelating capacity of $60.20 \pm 0.63\%$. 17 peptides were identified by UHPLC-ESI-MS/MS, 9 peptides from β -lactoglobulin (APL, KGL, KID, IIAE, DISL, IVTQ, SFNPT, NSAEPE, NKVLVL) and 8 peptides from α -lactoalbumin (KIL, KLD, INY, AIVQ, SLPE, GGVSLPE, KKILDK, LDDDLTD). The antioxidant capacity of the peptides has been related to the prevalence of hydrophobic amino acids such as Ala (A), Pro (P), Val (V), Ile (I), Leu (L), Phe (F), Tyr (Y). Also, the presence of Lys (K) can contribute significantly to antioxidant capacity. Some of these amino acids were found in the peptides identified in the hydrolysate obtained under optimal conditions. In conclusion, the antioxidant activity of the hydrolysates may be related to the presence of specific amino acids within the peptide sequence. The composition of these hydrolysates and their biological activity can be affected by the physicochemical parameters applied during the hydrolysis process, such as the enzyme-substrate ratio and time.

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Flavonoids and Ellagitannins Characterization, Antioxidant and Cytotoxic Activities of *Phyllanthus acuminatus* Vahl

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Introduction

Phyllanthus acuminatus belongs to the most diversified genus of Phyllantaceae family, which is widespread globally, and comprises circa 14 different species in Costa Rica.

The phenolic composition of leaves from *Phyllanthus acuminatus* L., a plant commonly used in Costa Rica as traditional medicine, was studied using UPLC-ESI-MS on an enriched phenolic extract.

Results

A total of 20 phenolic compounds were identified, comprising eight flavonoids (two flavanones—pinocembrin isomers and six derivatives from apigenin, chrysin, quercetin, and kaempferol); seven ellagitannins, two flavan-3-ols (prodelphinidin B dimer and (epi)gallocatechin); and three phenolic acids (ellagic acid, trimethylellagic acid, and ferulic acid). All of these compounds are reported for the first time in *P. acuminatus*, while previously reported in the genus *Phyllanthus*.

Antioxidant evaluation was performed for *P. acuminatus* phenolic extract obtaining DPPH results with a remarkably low IC₅₀ value of 0.15 g/mL. Also, cytotoxicity on gastric AGS and colon SW20 adenocarcinoma cell lines was evaluated, and highly promising results were obtained, with IC₅₀ values of 11.3 g/mL and 10.5 g/mL, respectively. Furthermore, selectivity index values obtained when comparing cytotoxicity on normal Vero cells was SI > 20 for both cancer cell lines, indicating a particularly high selectivity.

Additionally, justicidin B, a metabolite extensively studied for its antitumoral activity, was isolated from a non-polar extract of *P. acuminatus*, and comparatively evaluated for both bioactivities. The DPPH value obtained for justicidin B was moderate (IC₅₀ = 14.28 g/mL), while cytotoxicity values for both AGS (IC₅₀ = 19.5 g/mL) and SW620 (IC₅₀ = 24.8 g/mL) cell lines, as well as selectivity when compared with normal Vero cells (SI = 5.4 and 4.2 respectively), was good, but lower than *P. acuminatus* extract.

Conclusion

These preliminary results suggest that *P. acuminatus* enriched phenolic extract containing flavonoids, ellagitannins, flavan-3-ols, and phenolic acids, reported for the first time in this plant, could be of interest for further cancer cytotoxicity studies to elucidate structure–bioactivity relationships, and the molecular mechanisms and pathways.

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Utilidad de las investigaciones etnomédicas para el uso seguro y eficaz de los productos herbarios

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Introducción

El objetivo de este trabajo es demostrar que los criterios de los pacientes sobre la utilidad de algunas especies botánicas para controlar sus enfermedades pueden aportar información útil para garantizar el uso seguro y eficaz de los productos herbarios.

Método

Sujetos adultos de ambos sexos (245,142 femeninos / 103 masculinos, edad 44 a 72 años) atendidos en los servicios de consulta externa y las salas de hospitalización del Instituto Nacional de Angiología y Cirugía Vascular entre Febrero y Abril de 2007 fueron incluidos de manera aleatoria en una encuesta en la que se indagó sobre la utilizaban de plantas medicinales

Resultados

Más del 80 % de los participantes en la encuesta utilizaban productos de plantas medicinales en forma de fitofármacos o preparaciones domésticas al menos ocasionalmente y confiaban en su eficacia para tratar diferentes enfermedades. Las fuentes fundamentales de información sobre el tema fueron sus familiares y amigos. *Allium sativum* y *Morinda citrifolia* fueron las más mencionadas entre las 22 especies botánicas citadas como útiles para el control de problemas circulatorios y de factores de riesgo de aterotrombosis como la diabetes, la hipertensión y la dislipidemia. Las evidencias científicas que pudieran sustentar las creencias de los pacientes fueron insuficientes o nulas en algunos casos. Existen reportes sobre efecto antiagregante plaquetario de preparaciones de algunas de las plantas mencionadas.

Discusión

Se ha puesto en evidencia un grupo de plantas utilizadas por los pacientes con enfermedades vasculares periféricas, pero es necesario potenciar las investigaciones para validar los efectos terapéuticos que se les atribuye.

Conclusión

Algunos productos de plantas pudieran ser útiles para el tratamiento de estas enfermedades. Sin embargo otros no son apoyados por suficiente evidencia. Acciones farmacológicas como la antiagregante plaquetaria que acompaña a ciertos productos sugiere que los pacientes necesitan de la orientación médica, científicamente fundamentada, para garantizar el uso seguro y eficaz de los medicamentos herbarios.

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Food sovereignty Wayuu: factors, adaptive mechanisms and early alerts associated with the traditional consumption of native foods

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Introduction

The department of La Guajira (Colombia), has approximately 900,000 habitants, of which about 40% belong to the Wayuu ethnic group, mostly settled in the municipalities of Uribia, Manaure, Maicao and Riohacha. Traditionally, this ethnic group has used native plants and their traditional knowledge as a food source to prevent and cure their diseases. However, in recent decades, this knowledge has been lost rapidly causing diseases and malnutrition to occur mainly in children. Associated with this problem are the erroneous policies of the Government, the interference of the *Alijunas*, loss of knowledge about native plants and the effects of climate change that affect the water supply and have weakened the adaptive mechanisms and early warnings that have used as survival tools in the environment where they live. The aim of this research was to know the current state of its food sovereignty and factors that have influenced the habits of traditional consumption.

Method

A quantitative ethnographic study was conducted, obtaining information *in situ* in 30 Wayuu communities about the consumption of food from native plants and factors that have influenced their adaptive mechanisms and early warnings related to their food sovereignty.

Results / Discussion / Conclusion

There were reported 37 native plants associated with their traditional consumption, of which the ahuyama, the corn and the beans are cultivated in the rozas and are characterized by their daily consumption, while the fruits of the cherry, olive, iguaraya and the trupillo are part of the traditional food diet of the Wayuu Indians. However, these plants are affected by factors such as lack of rainfall, long periods of drought and loss of consumption habits of native plants, the main factor being the introduction of Western culture as one of the causes of loss of traditional knowledge. Therefore, it is necessary to implement comprehensive strategies that involve Wayuu Traditional Authorities and the strengthening of adaptation mechanisms and early warnings associated with food consumption.

Key words: Wayuu, resilience, traditional consumption, food sovereignty.

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***Pistacia lentiscus*: a natural source of bioactive molecules for the treatment of diabetes and inflammatory-related disorders**

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Introduction

Medicinal plants are believed to be an important source for the discovery of potential antioxidant, anticancer, anti-inflammatory and anti-diabetic substances. The present study was designed to investigate the anti-inflammatory, antidiabetic, anti-hyperuricemic and anticancer potential of *Pistacia lentiscus* (Anacardiaceae) extracts, as well as identification of active compounds, using appropriate methodology. Evaluation of antioxidant activity was undertaken to support its anti-inflammatory effects.

Method

The antioxidant potential of plant extracts against known radicals was measured using various standard *in vitro* methods. Anti-inflammatory activity was determined using the paw edema model in mice whereas the anti-diabetic effect was assessed *in vivo* by streptozotocin (STZ)-induced diabetic rats and *in vitro* by inhibition of alpha-amylase. Anti-inflammatory activity of plant extracts using *in vitro* assay was equally evaluated by measuring the secretion of pro-inflammatory cytokine (Interleukin-1 β) using ELISA kits. Diuretic activity was assessed by measurement of urinary volume output and determination of electrolytes (Na⁺ and K⁺). The anticancer activity of *P. lentiscus* extracts was equally investigated on human cancer cell lines. Identification of phenolics was also carried out using HPLC-MS.

Results/Discussion/Conclusion

Plant fractions exhibited high scavenging capacity against DPPH, NO[•] and ABTS⁺ radicals in a dose-dependent manner, with respective IC₅₀ values of 4.6, 2.71 and 1.0 μ g/ml and restored blood glucose levels, *in vivo*, to normal values, in agreement with the *in vitro* anti-diabetic effect (65% inhibition of α -amylase). Oral administration of plant extracts significantly decreased (60% inhibition) carrageenan-induced mice paw edema, similar to the standard drug, diclofenac, was effective in reducing the serum levels of IL-1 β in cell culture and induced a significant increase in urinary volume in mice, associated to a promising anti-hyperuricemic activity. *P. lentiscus* extracts were also found to be significantly cytotoxic on melanoma B16F10 cell line. HPLC-MS analyses allowed the identification of known and new phenolic compounds that

could be responsible for the observed activities. Therefore, *Pistacia lentiscus* extracts could be beneficial in the treatment of inflammatory conditions and diabetes complications.

Acknowledgements

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Antitumor effect *in vitro* and *in vivo* against a murine mammary adenocarcinoma model was induced by *Rhopalurus junceus* scorpion venom

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Introduction

In Cuba the endemic scorpion species *Rhopalurus junceus* has been used in traditional medicine for cancer treatment and related diseases. However there is no scientific evidence about its therapeutic potential for cancer treatment. The aim of the study was to determine the antitumor effect of scorpion venom against a murine mammary adenocarcinoma F3II.

Materials and Methods

The cytotoxic activity was determined by MTT assay with venom concentrations ranging from 0.1–1 mg/ml. Apoptosis was determined by RT-PCR and flow cytometry. Toxic effect in healthy animals and tumor growth kinetics in F3II bearing-mice were evaluated by using scorpion venom doses by the intraperitoneal route.

Results

Scorpion venom induced a significant cytotoxic effect ($P < 0.05$) in F3II cells in a concentration dependent manner. The cell death event involves the apoptotic pathway due to up-regulation of proapoptotic genes (p53, bax), down-regulation of antiapoptotic gene (bcl-2), and 33% of Annexin V+/PI- cells at early apoptosis and 10.21% of Annexin V+/PI+ cells at late apoptosis. Scorpion venom induced significant inhibition of tumor progression ($P < 0.05$) in F3II bearing-mice in a dose-dependent manner. The antitumor effect was confirmed due to dose-dependent reduction of Ki-67 and CD31 proteins present in tumor tissue.

Discussion

Improvement of cancer treatment involves the search for novel natural sources with anticancer potential. Scorpion venoms have shown to be suitable natural sources due to their potentialities as anticancer agents (1, 2). Similar results were reported for *R. junceus* scorpion venom against human epithelial cancer

cell lines, including breast cancer (3) suggesting common targets in both human and murine tumors. Besides, our report corroborates the selective effect of this scorpion venom against cancer cells similar to other species (4). All this evidence suggests the potential of scorpion venoms for solid tumor therapy.

Conclusion

Evidence indicates that scorpion venom can be an attractive natural product for deep investigation and developing a novel therapeutic agent for breast cancer treatment.

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Protagonism of animals in the Wayuu pharmacopea

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Introduction

The use of plants for therapeutic purposes has always played an important role in the life of the human being, and currently maintains a wide validity despite the enormous technological advances and the pharmaceutical industry. However, there are other elements that are used and play a leading role in the Wayuu Pharmacopoeia for the treatment of diseases, these are the animals and minerals that predominate in the in the forest of La Guajira. The aim of this research was to know the use of animals, minerals and derived compounds in the prevention and cure of symptoms and diseases that affect the Wayuu indigenous communities in the department of La Guajira.

Method

A quantitative ethnographic study was carried out through formal interviews with the piaches and healers located in the municipalities of Uribia, Maicao, Riohacha and Manaure, obtaining argued information of the animals, derived compounds, types of diseases, treated symptoms and preparations .

Results / Discussion / Conclusion

There were reported 52 species of animals (birds, crustaceans, insects, mammals, fish and reptiles) involved in the cure and prevention of diseases of Wayuu Indians, as well as 27 products represented in minerals and derived compounds (red bija, Ulisha stone, Salt of Epsom, goat tallow, termite nest, chicken fat, rattlesnake, duck feathers, among others), related to the prevention and cure of 40 diseases. It is concluded that the Wayuu communities within their traditional medicinal knowledge make use of a large number of animals and derived compounds to prevent and cure the symptoms and diseases that affect them, such knowledge is part of their cultural identity that has lasted for many years before the absence of health centers in their natural environment.

Key words: animals, wayuu, pharmacopoeia, ethnographic study, diseases.

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Zeolites. Its utility in feeding ornamental birds

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Abstract

The proper feeding of ornamental birds is important. In the search for a food product that was economical and available, the use of the product developed by the Research Center for the Metallurgical Mining Industry (CIPIMM) arose, under the Zeonut brand made with natural zeolites and minerals in specific concentrations for these species. On these bases, a study was carried out in which the effectiveness of said product as a dietary supplement of these birds was evaluated, in which 60, 30 personatas and 30 agapornis were included and distributed in two homogeneous groups, to one (Group C) The usual food was offered and the other was offered ZEONUT ad libitum. The results showed that all the animals accepted the administration of the product, they did not show changes in their eating habits, the excreta maintained their characteristics in terms of consistency, they did not have unpleasant odors or changes indicative of digestive disorders, the animals increased their body weight, the plumage was brighter in those who consumed the product, so the administration of the product was effective in the samples studied and it is recommended to continue these studies in a larger sample and in other species.

Key words: ZEONUT, ornamental birds, feeding



Zeolite. Its usefulness in the treatment of dermal and otic diseases of affective animals.

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Abstract

The dermal and otic diseases of domestic canines and felines are frequent and very difficult to treat. Different pharmacological products are used as a conventional practice, many of them are imported. In search of therapeutic options that are effective and economic, the use of the product developed by the Research Center for the Mining and Metallurgical Industry (CIPIMM), under the brand DEOSEC-F, powder mineral base micronized natural aluminosilicates, arose and modified with effective substances for the control of fungi, bacteria and is drying. On these bases, a study was carried out in which the effectiveness of said product in the treatment of dermal and otic diseases in domestic canines and felines was evaluated in a sample composed of 30 dogs and 10 adult cats of different ages, sexes and races. Two equal groups were

defined consisting of 15 dogs and 5 cats each: which presented skin, eyelid and ear conditions. Control group (C) was recommended the conventional treatment and treatment group (T) consisted in sprinkling the talc DEOSEC-F every 12 hours on the skin and the ears, until the recovery. The results showed that the treatment with DEOSEC-F was effective, being faster (3-7 days) in comparison with the conventional one that usually lasts up to weeks, and 100% of the animals of this group responded to the treatment, no secondary reactions or toxicities were observed, it was very easy to apply and well accepted by the animal and the owners. It was recommended to introduce the result in a larger sample in time and species.

Key words: DEOSEC-F, dermal diseases, otic diseases, canines, felines.

Zeolites Its usefulness in the treatment of surgical wounds in female dogs

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Abstract

Mammary tumors are frequent in bitches. The treatment is generally surgical, complex. Surgical wounds in these cases are wide. It is difficult to find hemostatic products useful in these situations. In search of therapeutic options that are effective and economic, the use of the product developed by the Research Center for the Mining and Metallurgical Industry (CIPIMM), under the brand DEOSEC-F, powder mineral base micronized natural aluminosilicates, arose and modified with effective substances to be hemostatic, antimicrobial and drying. On these bases, a study was carried out in which the effectiveness of said product in the treatment of superficial haemorrhages which are produced in cases of surgical operations of mammary tumors and was evaluated in a sample composed of 60 young and female adult dogs of different ages, sexes and races. Two equal groups were defined consisting of 30 bitches each one. Control group (C) no was recommended treatment and treatment group (T) consisted in sprinkling the talc DEOSEC-F in the wound until the bleeding was stopped. The results showed that the treatment with DEOSEC-F was effective, hemostatic effect was produced in 2-3 minutes. In control group, hemostasis delay 4-5 minutes. No secondary reactions or toxicities were observed, it was very easy to apply. It was recommended to introduce the result in a larger sample in time and species.

Key words: DEOSEC-F, haemorrhagies, hemostatic, dogs.

Tincture of guava in the treatment of pigs with diarrheal syndrome

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This work was carried out at the UEB La Caoba Swine Multiplier Center in Villa Clara Province with the objective of determining the evolution of guava tincture in porcine diarrheal syndrome. For the development of the same 20 pigs of the categories of pups and precebas were used that were divided into two groups, first 11ml of 20% guava tincture was administered with a frequency of three times a day orally, for 72h. A similar amount of product was administered to the second group but at a concentration of 1% determined as a control group. For the statistical analysis of the results, the data used in the Excel computer program were collected and the statistical package STADGRAPHIC (version 4.1) was used, and a hypothesis test was used to compare proportions. In the analysis of the data of the first follow-up consultation, it can be said that the 20% paint had a significantly higher antidiarrheal effect than the 1% paint because it recovered 20% of the animals, while with the latter there was no case recovered. This trend became increasingly noticeable and significant, a favor of the group treated with 20% paint, so that the amount of animals recovered is 5 times greater at 48 h.

Keywords: guava tincture, diarrheal syndrome, pig

Physicochemical and bioactive properties of twenty honey samples from various floral origins from Guerrero, Mexico

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Honey is a natural sweet substance produced by honeybees through foraging of different plants, transforming the collected material in their bodies into a liquid and depositing the liquid in the cells of honeycombs. The properties of honey are shaped to a large extent by the plants foraged by the honeybees (Beretta, 2005). Most studies on the effects of honey are concentrated on the activities of bioactive compounds, especially phenolic compounds, in the human organism. The most widely distributed in nature, include the phenolic acids and flavonoids (Vermerris & Nicholson, 2006). Several authors also studied the correlations between color and antioxidant and antibacterial activities with content of the bioactive compounds of honey (Beretta, 2007). Recent studies try to explain the relation between phenolic content and color of the honey, indicating that phenolics, proteins and sugars in the honey may form high molecular weight melanoidins, multi-component polymers which exhibit antioxidant properties (Brudzynski & Miotto, 2011a, 2011b)

Physicochemical and bioactive properties in Mexican honeys are limited, therefore, it is currently very important to determine parameters in honey samples from the state of Guerrero, Mexico, especially due to the high productive and economic relevance on the Mexican honey market. Current analysis assessed the antioxidant activity and the content of bioactive compounds in 20 honey samples.

The present study was accomplished to determine the physicochemical, and antioxidant activities of Mexican honey samples, Total phenolic content, antioxidant activity (two assays: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and ferric reducing antioxidant power (FRAP)), and Color of honey samples were determined using Honey Color Analyzer C 221 (Hanna Instruments, Rhode Island, and USA). Honey color is defined as millimeters on a Pfund scale for optical density with glycerol as reference.

Twenty mexican multifloral honeys were evaluated for several batches of the same honey type and statistically analyzed. Total phenolic content (121.6 a 1173.8 mg GAE/kg) it was correlated with the color parameters as well as antioxidant activity. The honeys' color ranged from pale yellow to dark amber. Dark "costa chica" honey with much higher phenolic content (1113.0 μ g GAE/g) exhibited higher antioxidant activity (4.9 mmol Fe²⁺/kg and 1.01 mmol TEAC/ kg). Color can be helpful for identifying and distinguishing between several honey types, such as "centro" region and "costa chica" region, but almost no

differences were found among very light honeys (r). This analysis revealed general characteristic of Mexican multifloral honeys with significant differences among the samples of other honey types.

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Contribution to cuban natural and traditional medicine, multidisciplinary experience for more than 15 years

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Abstract

For more than 15 years, a multidisciplinary group pays tribute to Natural and Traditional Medicine, with one essential objective: to obtain a Cuban natural product in order to improve the quality of life of women. In response to the updated national and international demands related to obtaining natural products with medicinal properties, ethnobotanical knowledge interacted in an integrated way; phytochemical and chemical analysis techniques; pharmaceutical quality controls; toxicology tests; preclinical experiences until technological trials of pharmaceutical preformulation. With a vision of a sustainable development that makes it possible to harmonize the biopharmaceutical industry with the environment, studies have focused on a species of native Cuban flora through the use of clean technologies, friendly to the environment and with guarantees of its cultivation and conservation. Their active principles and biological activity are detected and identified; crops are established under *ex situ* conditions, including field plantations of medicinal plants; the raw material, its parameters of quality and adequate dose are defined; systematic assessments of stability and chemical characterization are described; there are preclinical and toxicological results of the raw material and a preliminary preformulation that fulfills the technological requirements and analytical quality standards. We are heading towards the registration of a Nutritional Supplement with antioxidant activity for climacteric women, in the first instance, and continue to reach higher phases that allow us to close the research - technological development - production - commercialization cycle.

Natural and Traditional Medicine, Nutritional Supplement, antioxidant, sustainable development.

The functional jelly rich in bioproducts

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Introduction

The consumption of functional foods is one of the main searches of the society in the 21st century. The consumer's growing demand for foods that, besides sensorial and nutritional qualities, have health benefits, gives rise to the need for new ingredients and formulations that can meet the current market. Plant hemicelluloses are partially hydrolyzed galactomannans which are biochemically classified as polysaccharides and can be used as food additive of low cost by its thickening function. They have functional properties and their consumption has been associated with benefits to human health as they are source of dietary fiber (Rodriguez, 2016). The Mojito is a classic cocktail enjoyed over 100 years in Havana made with blend of native ingredients of the Caribbean such as mint and lemon. The aim of the work was to elaborate a functional jelly rich in galactomannans flavored with bioingredients of Mojito non-alcoholic cocktail.

Method

The galactomannan extraction was carried out from the endosperm of the flamboyant (*Caesalpinia Pulcherrima*) seeds (Cerqueira et al., 2009) in the Pharmacology laboratory and in the Nucleus of Experimental Biology from University of Fortaleza (UNIFOR). Afterword, in the Nutrition and Dietetics laboratory (UNIFOR), pre-tests on galactomannan concentrations were made for the production of jelly (Lira Filho, 1995). Functional jelly formulations (GM1 and GM2) were elaborated in the Kitchen Laboratory of Federal Institute (IFCE), Baturité campus using bioingredients of Mojito non-alcoholic cocktail and galactomannan as fiber source and food thickener. Both samples were used for sensorial analysis (Acceptability Test) performed with untrained tasters. The organoleptic properties were evaluated through the hedonic scale.

Results/Discussion/Conclusion

The organoleptic properties for both samples were promptly appreciated for the tasters revealing a preference for the most thick jelly (GM1). The citrus and refreshing blend of Mojito's Jelly is a functional food composed primarily of mint, lemon juice, passion fruit bark and fibers (galactomannans). For the development of this new food product the choice of the thickening and emulsifying agent galactomannan was due to the availability of *Caesalpinia Pulcherrima* seeds, common in the northeast region of Brazil. The galactomannans as a alternative source of dietary soluble fibers are requested for a health diet. It must be said that galactomannans not change the jelly flavor. The functional Mojito's Jelly represents a technological innovation and reveals as a good candidate in the nutritional market for bioproducts. It is important to point out the need for further research to arrive the ideal formulation so that it can be commercialized.

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Challenges of innovation in Brazilian herbal medicine: a study on the development of national patents

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Introduction

Brazil is a megadiverse country with about 20% of the world's biodiversity, which allows the country to have effective participation in the market of innovative bioproducts such as herbal medicines. LEPESH-CUNHA et al. (2018) investigated a sample of 85 species of Amazonian occurrence of strategic interest for the development of products. They verified that Brazil presented the largest production of scientific articles in the biological, chemical, biochemical, medicine, biomedicine, pharmacy, toxicology, immunology, biotechnology and microbiology fields. Despite the great potential for the use of biodiversity in Brazil, there are still few successful cases in patent protection of herbal medicine inventions. França & Vasconcellos (2018) identified all patent applications of herbal medicines of Brazilian origin done in Brazil in the period from 1995 to 2017 (n=876) and verified that only 1.3% (12) had been granted. The objective of this work is to analyze the 12 patents of herbal medicines of Brazilian origin that were granted by the Brazilian INPI and to verify if the products associated with them have become innovations in the market.

Method

Patents for herbal medicines granted to nationals in Brazil in the period 1995-2017 were identified from the work of França & Vasconcellos (2018). The documents were selected on the basis of groups A61k35/78 and A61k36/... (preparations for medical, dental or hygienic purposes and, more specifically, medical preparations containing undetermined materials or their reaction products, derived from plants) of the International Patent Classification (IPC). The contacts of the owners of these 12 patents were identified through search on the Platform Lattes and Google to identify the success stories in which the product associated with the patent has reached the market. Moreover, in cases where the invention did not reach the market, the difficulties encountered by patent owners were also identified.

Results/Discussion/Conclusion

Based on the analysis of herbal medicines granted patents, three were identified that became innovative products in the market: (1) Patent PI0406165, "Regenerating product for eliminating stretch marks or other derogatory occurrences of natural skin patterns comprising a mixture of oils, retinol, vitamin E and collagen, and process for application of regenerating product" has been submitted by an independent inventor and it continues to be developed in Brazil. Another patent developed was (2) Patent PI0204130, entitled "Gel composition based on Pothomorphe umbellata extract, its uses and non-therapeutic cosmetic method", this patent was licensed by the Natura company, being the first patent of the University of São Paulo (USP) to reach the market. It is important to note that this product is no longer on the market due to competitive issues which led to the production line being discontinued by the company. An equally important third process patent that came to the national market was the (3) Patent PI1003060, "Process for obtaining partially purified extracts of antioxidant compounds from fruits of palms of euterpe genus", this was licensed and developed by the company Amazon dreams. These results show how much scientific and technological efforts are needed to generate an innovation in herbal medicines in Brazil and other factors, as good market strategies are important for the permanence of these products in the Brazilian market that already shows itself competitive in this area.

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Flavonols and antioxidant activity of *Fumana thymifolia*

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Introduction

The genus *Fumana* (Dunal) Spach (Cistaceae) is represented by about 25 perennial species in the Mediterranean flora [1]. Usually, these species are growing on dry, rocky, stony or sandy habitats within two major centers of biodiversity, the western and eastern Mediterranean [2]. The chemistry of *Fumana* genus has not been well investigated even though the studies conducted to date report the presence of different natural products including essential oil [3], polyphenols and flavonoids [4]. The phytochemical investigation of the ethyl acetate extract of *Fumana thymifolia* resulted in the isolation of seven flavonoids (**1–7**) and a biflavonoid (**8**). Additionally, the antioxidant activity of crude extracts (EtOAc and *n*-BuOH) of *Fumana thymifolia* was evaluated by DPPH free radical-scavenging method. In further, the total phenolic and flavonoid contents were determined using Folin–Ciocalteu and trichloroaluminium technics.

Method

1 kg of dried plant *Fumana thymifolia* was extracted with EtOH–H₂O (70:30, V/V) for 48 h, three times. After filtration, the filtrate was concentrated and dissolved in H₂O (600 mL). Suspension was extracted successively with petroleum ether, ethyl acetate and *n*-butanol. The organic phases were dried with Na₂SO₄, filtered and concentrated to obtain the following extracts: petroleum ether (5 g), ethyl acetate (14 g) and *n*-butanol (41 g). The ethyl acetate extract (7 g) was submitted to vacuum liquid chromatography (VLC) on silica gel RP-18 using H₂O–MeOH (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90) and methanol, to obtain 16 fractions of 100 mL combined together into five total fractions. Fraction F₁ (3.7 mg) subjected to purification by SiO₂ column using a gradient CHCl₃–methanol (98:2, 96:4, 92:6, 90:10, 85:15, 80:20, 70:30) to afford eighteen sub-fractions. After repeated polyamide column chromatography (gradient toluene–methanol) and by using of HPLC, sub-fraction 9 (124 mg) gave pure compounds **8** (8 mg), **7** (10 mg) and **6** (7 mg). Subfraction 10 (421 mg) was purified on polyamide column and preparative RP-18 TLC (H₂O–MeOH 7:3) to afford compounds **1** (6 mg) and **2** (7 mg). Subfraction 11 (178 mg) was also chromatographed by polyamide column to afford compounds **3** (5 mg) and **4** (6 mg). From subfraction 12 (74 mg) pure compound **5** (10 mg) was obtained by precipitation in MeOH.

Results/Discussion/Conclusion

The structures of the isolated compounds were determined on the basis of 1D and 2D homo- and heteronuclear NMR and mass spectrometry, as well as by comparison with reported literature data. In fact, this chemical study allowed the isolation and characterization of eight compounds: 3-methoxy-7-*O*- β -glucopyranoside kaempferol (**1**), 3,4'-dimethoxy-7-*O*- β -glucopyranoside quercetin (**2**), Isoquercitrin (**3**), 3-methoxy-7-*O*- β -(6"-galloylgluco-pyranoside) kaempferol (**4**), 3-methoxy-7-*O*- β -(6"-galloylglucopyranoside) quercetin (**5**), iriflophenone-2-*O*- β -glucoside (**6**), gallic acid (**7**) and dihydrodaphnodorin B (**8**). It should be noted that this is the first report of compound (**6**) in Cistaceae family.

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Cervical intraepithelial neoplasia, in women under 25 and up to 30 years. MTN treatment

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Summary

Cervical intraepithelial neoplasia is a lesion that is considered the prelude to uterine cervical cancer. Human papilloma virus (HPV) infection in the genital tract, especially some subtypes called oncogenic, plays an important role in the occurrence of intraepithelial lesions and their possible evolution to invasive cancer in these locations. There is also an important group of risk factors or cofactors that, associated with HPV infection, can cause these cellular changes in the cervix. A descriptive quantitative cross-sectional study was carried out, in the period from January 2011 to June 2018; with the objective of describing the risk factors related to cervical cancer present in adolescent girls who attend the neck pathology clinic of the Dr Luis Díaz Soto Hospital. In this paper, these risk factors and the appearance of cervical lesions in younger and younger women are analyzed. The issue of treatment with medicinal plants of some lesions, such as Aloe Vera, Manzanilla and Llantén, is addressed.

Keywords: Women under 25 and up to 30 years, human papilloma virus, uterine cervical cancer.

Producción de acetato de lupeol a partir de un cultivo de células en suspensión de *Cnidoscolus chayamansa*

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Introducción

C. chayamansa (Euphorbiaceae) es apreciada por su valor nutricional y medicinal. Es un arbusto cultivado en la región maya desde Guatemala, Belice, sureste de México y parte de Honduras. Como remedio tradicional es empleada para tratar el cáncer, diabetes mellitus, diurético, trastornos gastrointestinales y en procesos inflamatorios. Recientemente, se ha reportado el efecto hepatoprotector y antiinflamatorio (modelo agudo y crónico) del extracto orgánico de las hojas de chaya;¹ así como, el efecto biológico de algunos metabolitos aislados de este extracto².

Dado su valor, se propuso evaluar distintos reguladores de crecimiento vegetal (RCV) para la obtención de callo a partir de explantes. Posteriormente, se establecieron los cultivos de células en suspensión a partir de los callos, por medio de una cinética de crecimiento se calcularon distintos parámetros como la biomasa máxima, tasa específica de crecimiento (μ), índice de crecimiento (GI), tiempo de duplicación (td). A partir de las células de esta suspensión, se obtuvo el extracto CHCl₃:MeOH vía maceración exhaustiva cuantificando el contenido de acetato de lupeol (AL) en el extracto.

Método

C. chayamansa se colectó en la CdMx y su identificación taxonómica se realizó en el herbario del IMSSM (ID 16252), varios ejemplares fueron depositados en el invernadero de UAM-I para su aclimatación por tres semanas. Se colocaron explantes de hoja aséptica en un medio de cultivo que contiene reguladores de crecimiento vegetal (RCV) para inducir la diferenciación del callo. Se utilizaron diferentes combinaciones de la auxina ácido α -naftalenacético (ANA) y la citoquinina 6-benzilaminopurina (BAP) a concentraciones de 2.5 y 5.0 mg L⁻¹. El callo friable se disgregó y colocó en un medio de cultivo MS para establecer el cultivo de células en suspensión por 40 d, se extrajo la biomasa para obtener el extracto CHCl₃:MeOH y realizar la cuantificación de acetato de lupeol (AL) por HPLC³.

Resultados/Discusión/Conclusión

Para la inducción de callo, se colocaron hojas inmaduras en medio MS suministrados con la auxina (ANA 2.5 mg L⁻¹) y la citoquinina (BAP 5.0 mg L⁻¹). El callo friable se utilizó para establecer un cultivo de células en suspensión y cuantificar la producción de AL. Por medio de una cinética de crecimiento con duración de 60 días se calcularon los siguientes parámetros: biomasa máxima a los 40 días = 9.2 gL⁻¹; GI = 5.63; μ = 0.082 días⁻¹ y td = 8.45 días. El crecimiento celular fue dependiente de la concentración de D-glucosa en el medio de cultivo. En los extractos secos (CHCl₃:MeOH) de los cultivos en suspensión celular a los 40 días se cuantifico el contenido de AL siendo de 38.1 mg AL g⁻¹ de extracto en células, valor que fue mayor a la concentración en las hojas de plantas silvestres (2.9 mg de AL g⁻¹ de extracto).

Para establecer un cultivo en suspensión celular a partir de hojas inmaduras de *C. chayamansa* se utilizó los reguladores del crecimiento vegetal ANA (2.5 mg L⁻¹) + BAP (5 mg L⁻¹) en medio MS suplementado con D-glucosa. Esta suspensión celular de *C. chayamansa* puede ser una fuente alterna para la producción de AL, ya que la producción máxima de AL en el cultivo fue de 38.1 mg AL g⁻¹ de extracto vs planta silvestre (2.9 mg de AL g⁻¹ de extracto).

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Citotóxicidad y actividad leishmanicida de diterpenos tipo *ent*-beyerenos

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Resumen

Leishmaniasis son un grupo de enfermedades zoonóticas y antroponóticas causadas por parásitos del género *leishmania*, y es transmitida a humanos por la picadura de *flebótomos* [1]. Se presenta como leishmaniasis cutánea (CL), la más común, mucocutánea (MCL) y visceral (VL) [2]. Los tratamientos incluyen antimoniales pentavalentes, anfotericina, pentamidina y miltefosina, sin embargo, estos medicamentos presentan graves efectos secundarios como hepatotoxicidad, trombocitopenia y nefrotoxicidad, además de requerir de largos periodos de tratamiento que a veces son ineficaces dado la resistencia por parte del parásito [3,4]. Por lo tanto, urge encontrar nuevas alternativas terapéuticas.

Existen algunos reportes de diterpenos activos contra *leishmania*, como abietanos [5] y labdanos [6], sin embargo, la actividad leishmanicida de diterpenos tipo *ent*-Beyerenos no ha sido reportada. En este trabajo evaluamos la actividad leishmanicida *in vitro* e *in vivo* de dos compuestos diastereoisoméricos, el *ent*-Beyer-15-en-18-ol y *ent*-Beyer-15-en-19-ol (beyerenol **1** y **2**) aislados de la especie *Bacharis tola* [7] **Figura 1**; además reportamos la hemisíntesis del *beyerenol 1* a partir del *rebaudiosido*, importante edulcorante utilizado en la industria alimenticia.

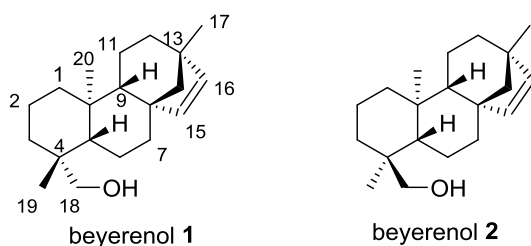


Figura 1

Los ensayos *in vitro* se realizaron sobre amastigotes intracelulares de la especie *L. (V) braziliensis* y la evaluación *in vivo* se utilizó el modelo de hámster dorado (*Mesocricetus auratus*) para leishmaniasis cutánea (CL).

El beyerenol **1** y **2** mostraron EC_{50} of 4.6 $\mu\text{g/mL}$ (16.0 μM) y 5.3 $\mu\text{g/mL}$ (18.0 μM) contra [amastigotes](#) intracelulares de *L. (V) braziliensis* respectivamente. Los experimentos *in vivo* mostraron cura del 50% sobre hámster infectados con *L. (V) braziliensis* mediante aplicación tópica, Crema **I** (beyerenol 1, 0.81%, w/w) y Crema **III** (beyerenol 2, 1.96%, w/w).

Los resultados de este trabajo sugieren que beyerenol **1** y **2** son potenciales candidatos para quimioterapia de leishmaniasis cutánea por aplicación tópica, además que la orientación del grupo hidroximetilo no es importante, ya que presentan una actividad similar. Sin embargo, este grupo funcional es indispensable, ya que su oxidación al respectivo ácido carboxílico afecta negativamente su actividad.

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Phytoconstituents, antioxidative and anti-inflammatory effect of extracts of *Malachra alceifolia* Jacq leaves from the Colombian Caribbean region.

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Introduction

Malachra alceifolia Jacq is one of the species of plants to the family Malvaceae, little studied in the phytochemical and ethnopharmacological. Traditionally this plant was used in the form of extracts, powder, paste by populations from the northern Colombian for treating fever, stomach, inflammations. The chemical phytoconstituents from leaves of *M. alceifolia* included flavonoids, terpenoids, glycosides, steroids, saponins, tannins, alkaloid and phenolic compound.

Methods

This study investigated the antioxidative and anti-inflammatory effect of bioactive compounds isolated of *M. alceifolia* on RAW264.7 mouse macrophages. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Measurement of nitric oxide (NO) synthesis was performed using the NO detection. Measurement of free radical scavenging activity was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The assays were done by triplicate in at least two independent experiments, as positive controls were used betamethasone, deoxycorticosterone acetate (DOCA), β -estradiol (1nM- 50 nM), quercetin and aminoguanidine 0.1 μ g/mL- 0.1 μ g/mL. Results were expressed as fifty cytotoxic concentrations (CC₅₀), concentration necessary to kill 50% of cells, and fifty effective concentrations (EC₅₀) calculated by The Analysis of Variance (ANOVA) used multiple comparison analysis statistics include the following tests Tukey and Dunnett. Program *Graph Pad Prisma* ver. 6 was used to analyze the data. Differences were considered statistically significant at $P < 0.05$.

Results/Discussion/Conclusion

The chemical analysis extracts of *M. alceifolia* of leaves evaluated revealed the presence of five bioactive fractions. The MTT assay revealed that bioactive fractions of *M. alceifolia* exerted no significant cytotoxicity in the RAW264.7 macrophage cells. These results suggest that the inhibition of NO production by *M. alceifolia* may reduce the tissue damage caused by the inflammatory reaction. Two fraction derived from the

CHCL₃ extract showed significant DPPH scavenging activity (IC₅₀: 70 and 150µg/mL) compared with the values obtained for quercetin (IC₅₀: 5µg/mL) standard. These results suggest that *M. alceifolia* can exert its anti-inflammatory and antioxidative effects probably by suppressing pro-inflammatory. This study was supported by the University of Cartagena, the Program to Support Research Groups, sponsored by the Vice-Rector for Research at the University of Cartagena (Grant RC 139-2017; Grant RC 054-2018). The National Program for Doctoral Formation Colciencias, 727- 2015 and Doctoral program in biomedical sciences from University of Cartagena.

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Antimicrobial activity and chemical composition of the hexanic extract of the leaves and stem bark of *Croton micradenus urb*

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Abstract

The Cuban flora possesses a considerable wealth and diversity of the native species (7500) and a high percentage of the endemism (51,4%). Inside this high vegetable diversity, it stands out the Euphorbiaceae family, alone not to be one of the most numerous, but also for the quantity and diversity of the chemical elements and applications that it possesses. In Cuba, the mentioned family is integrated by 38 genera, among which *Croton* stands out so much for the number of species that compose it (53), of them 37 endemics that represent 69,8%, like for the reports as for the uses in the traditional medicine of the same ones. To mention some examples, their effectiveness like diuretic, antimalarial and antimicrobials. Isolated compounds of the hexanic extracts of species of the family have investigative interest for the shown biological activity, among them *Croton campestris* and *Croton scabiosus* to exhibit activity in front of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. In such a sense, we intended to be carried out and to report the chemical characterization and the antimicrobial activity of the hexanic extract of *Croton micradenus Urb.* Cuban endemic species that grows in the coastal area Maisí-Guantánamo, by means of CG-MS and pharmacological assays. *C. micradenus* showed antimicrobial activity in front of *Staphylococcus aureus* and *Escherichia coli*, not being this way, in front of *Pseudomonas aeruginosa* and *Candida albicans*. The presence of octacosanol, palmitic acid, oleic acid and stigmasterol, it confers its responsibility on the activity expressed by the species.

**Phytochemistry, pharmacology and pharmacognosy



Enrichment of apple juice with bioactive compounds from selected plant materials and their by-products

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Introduction

Current trends of healthy life style have important input on the food industry. Consumer preferences of choosing ready and easy to drink immediately beverages make liquid or semiliquid products desirable. Juices are increasingly popular way of consuming fruits and the industry is focusing on the maintenance the content of bioactive compounds. In this study, phenolic profile, antioxidant and antidiabetic activity, colour, vitamin C, sugar and organic acid content of six new apple juices enriched with strawberry-tree and persimmon fruits, myrtle berries, feijoa flowers or saffron petals juice were investigated. Moreover, their sensory attributes were determined.

Method

Polyphenols in analysed juices were identified by LC-PDA-QToF/MS and quantified by UPLC-PDA-FL (Wojdyło et al., 2013). Determination of sugar content using HPLC-ELSD, organic acid and vitamin C content, as well as physicochemical properties (colour, dry residue, ashes, TSS and TA) were evaluated. Moreover, antioxidant activity assays (FRAP, CUPRAC, DPPH[•], ABTS^{•+}, ORAC), total phenols by Folin-Ciocalteu method (Tuberoso et al., 2013) and digestive enzymes inhibition activity assays (α -amylase, α -glucosidase and pancreatic lipase) using spectrophotometric methods were performed (Podsędek et al., 2014). Finally, sensory proprieties (colour, aroma, taste, consistency and desirability) of juices were evaluated by ten trained panelists using 5° hedonic scale.

Results/Discussion/Conclusion

Obtained results showed that supplementation of apple juice with strawberry-tree fruits have a positively significant influence on the increment of vitamin C (23.68 mg/100g of fw), as well as on consumer evaluation. Furthermore, strawberry-tree fruits and feijoa flowers enrich new juices in sugars (14.85 g/100 g fw) and organic acids (1.57 g/100 g fw), that may guarantee no sweetener addiction or better preservation of the product. LC-MS analyses confirmed presence of different phenolic compounds (anthocyanins, hydroxybenzoic and hydroxycinnamic acids, flavan-3-ols, flavonols and dihydrochalcones). Regarding each



particular product, the most represented phenolic compounds were quercetin derivatives, chlorogenic and cryptochlorogenic acids, cyanidin and delphinidin derivatives. The highest total polyphenol content (194.06 mg GAE/100 g fw), as well as the highest α -amylase and α -glucosidase inhibitory activity (61.37 and 15.90 mg of fw/mL, respectively) was evaluated in juice enriched with persimmon fruits. Regarding antioxidant activity, the highest values were observed in products containing persimmon fruits and feijoa flowers (2.96 and 3.51 mmol Trolox/100 g fw, ORAC). Obtained results suggest that the antioxidant activity of the juices is positively associated with the presence of total polyphenols (Pearson correlation = 0.9041, 0.9339, 0.8971, 0.8974 and 0.9081 for CUPRAC, FRAP, DPPH[•], ABTS^{•+} and ORAC, respectively). To sum up, the enrichment of commonly known juices with various plant materials and their by-products could be interesting from both a nutritional and commercial point of view, because of its positive influence on biological activity.

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Modelado molecular de la interacción de cardenólidos de la planta medicinal *Asclepias subulata* con la mutante HP53-Y220C

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Introducción

El factor de transcripción p53 es un homotetrámero que funciona como supresor tumoral y puede ser activado por estrés celular, inhibiendo la progresión del ciclo celular. La estructura del dominio central (p53C) es la más estudiada, ya que el 40% restante se encuentra desplegada (Freed-Pastor & Prives, 2012). Los niveles de p53 aumentan durante la progresión de tumores y cerca del 50% de estos se han asociado con mutaciones, afectando la formación del tetrámero y su capacidad supresora de tumores. p53-Y220C es la mutación más frecuente en p53C, fuera de la superficie de unión al ADN y tiene un fenotipo sensible a la temperatura que se asocia con 75,000 nuevos casos de cáncer (Joerger & Fersht, 2007). p53-Y220C reduce la estabilidad termodinámica de la proteína al formar una hendidura accesible al disolvente que conserva el plegamiento. Es necesario el diseño de fármacos estabilizadores para p53-Y220C donde el compuesto llene la hendidura, interaccionando con C220. Proponemos como fármaco natural para p53-Y220C los cardenólidos de *Asclepias subulata*, una planta angiosperma perenne nativa del desierto del noroeste de México y el suroeste de los Estados Unidos (Brewer Fernández, *et al.*, 2008). Existe evidencia etnofarmacológica de que las poblaciones nativas de Seris y Pimas han usado extractos de *A. subulata* para el tratamiento de enfermedades como el cáncer (Alonso-Castro, *et al.*, 2011). Recientemente, Rascón-Valenzuela *et al.*, (2016) evaluaron el efecto de cuatro cardenólidos de *A. subulata* en líneas celulares murinas y humanas, los cuales mostraron actividad antiproliferativa contra las líneas celulares humanas. Sin embargo, no se ha elucidado el mecanismo protector de los cardenólidos contra la mutación de p53-Y220C. En la presente investigación, se realizaron ensayos de docking de p53-Y220C con los cardenólidos de *A. subulata* para evidenciar las posibles interacciones, destacando su potencial uso farmacológico.

Método

El docking se realizó para predecir la unión de los cardenólidos de *A. subulata* a la hendidura de p53-Y220C, con MOE v2018 (Inc., C. C. G. 2016). Se seleccionó la estructura de p53-Y220C (PDB: 5O1A) como receptor. Los ligandos fueron 12,16-dihidroxicalotropina, calotropina, corotoxigenina-3-O-glucopiranosido y desglucuzarina. Se calcularon las cargas parciales con CHARMM27 para el receptor y el campo de fuerza MMFF94x para los ligandos (Mackerell *et al.*, 2004). Se realizaron tres series estocásticas para obtener las 30 poses con las mejores puntuaciones, que son sometidas a un refinamiento estructural con el protocolo de

ajuste inducido. Las mejores poses se seleccionaron de acuerdo con su puntuación y potencial uso como estabilizador de p53-Y200C. Para hacer una comparación entre p53-Y220C y p53 nativa, se utilizó PDB: 5MCU.

Resultados/Discusión/Conclusión

Las estructuras p53C nativa y p53C-Y220C tienen un RMSD de 0.29 Å, su principal diferencia está en la región de C220. p53 nativo no tiene esta hendidura ya que ese espacio está ocupado por Y220. De los 4 ligandos evaluados; 12,16-dihidroxicatropina y desglucuzarina no se unen a la hendidura de p53-Y220C, por que no son potenciales activadores. El ligando 9HG presente en p53-Y220C (PDB: 5O1A) se encuentra dentro de la hendidura también ocupada por los cardenólidos corotoxigenina3-O-glucopiranosido y calotropina. Los residuos que tienen contacto con los cardenólidos corotoxigenina3-O-glucopiranosido y calotropina fueron V147, T150, P151, C220, G221, P222, P223 y T230, que tienen interacciones hidrofóbicas con los ligandos. En dos de las cuatro poses para calotropina se encontraron puentes de hidrógeno con el residuo C220. Evidencias previas indican que corotoxigenina 3-O-glucopiranosido y calotropina participan en la inhibición de la proliferación de células cancerosas. Por lo que corotoxigenina 3-O-glucopiranosido y calotropina son potenciales activadores de p53-Y220C, debido a la estabilización de hendidura Y220C. La unión en la hendidura es impulsada por interacciones hidrofóbicas. Se evidencia que corotoxigenina 3-O-glucopiranosido y calotropina son potenciales fármacos para combatir el cáncer asociado con la mutación p53-Y220C.

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Participación del núcleo septal lateral en el efecto antidesesperanza del extracto metanólico de *Argemone mexicana* y *Verbesina persicifolia*

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Introducción

En México, la depresión afecta cerca del 14% de la población y es predominantemente femenina. Aun cuando, los tratamientos farmacológicos antidepresivos son eficaces, estos se acompañan de efectos colaterales y reacciones adversas que limitan su uso, aunado al alto costo que representan. Por lo que es necesario la implementación de terapias alternativas, tal es el caso de la herbolaria, que utiliza plantas con potencial antidepresivo, como *Verbesina persicifolia* y *Argemone mexicana*, dos plantas de diferentes familias y géneros a las cuales se les atribuyen efectos similares como analgésicos, ansiolíticos, sedantes y antidepresivos. Sin embargo, es necesario identificar las familias de metabolitos responsables de los efectos mencionados, además de conocer los mecanismos subyacentes y las estructuras cerebrales involucradas. Por otra parte, el núcleo septal lateral es una estructura límbica involucrada en las emociones, la respuesta al estrés y en las acciones de los fármacos antidepresivos, los cuales incrementan la frecuencia de disparo de las neuronas septales. Por lo tanto, en el presente trabajo se evaluó la participación del núcleo septal lateral en el efecto antidesesperanza del extracto metanólico de *Verbesina persicifolia* y *Argemone mexicana*.

Método

En una primera etapa se realizó un perfil fitoquímico basado en pruebas coloridas para la identificación de las familias de metabolitos presentes en los extractos metanólicos. Para la fase conductual se emplearon 28

ratas ovariectomizadas de la cepa Wistar distribuidas en 4 grupos que recibieron por vía intraseptal los diferentes tratamientos ensayados (Vehículo 0.6µl, extracto metanólico de *Argemone mexicana* 0.12µg/0.6µl, extracto metanólico de *Verbesina persicifolia* 0.12µg/0.6µl y Fluoxetina 0.5µg/0.6). Inmediatamente después de la administración, los animales se sometieron a la prueba de actividad locomotriz en campo abierto con el objetivo de descartar algún efecto motor (hipo o hiperactividad). Posteriormente los animales fueron evaluados en la prueba de nado forzado. Al finalizar los experimentos conductuales se extrajeron los cerebros para verificar el sitio de inyección. Para el análisis estadístico sólo se incluyeron los datos provenientes de los animales en los que la cánula guía se localizó en el septum lateral.

Resultados/Discusión/Conclusión

Los extractos metanólicos de Argemone y Verbesina presentaron un efecto antidesesperanza similar al obtenido por el antidepresivo Fluoxetina, cuando se administra directamente en el núcleo septal lateral, por lo que esta estructura podría estar involucrada en los efectos antidesesperanza producidos por los extractos metanólicos de Argemone y Verbesina. Se realizó un tamiz fitoquímico al extracto de ambas plantas, encontrando familias de metabolitos secundarios como taninos, quinonas, chalconas, cumarinas y leucoantocianinas en *Verbesina persicifolia* y ácidos grasos, esteroides, taninos y glicósidos en *Argemone mexicana*, además en ambos extractos se identificaron flavonoides/flavonas y alcaloides siendo estos últimos dos los posibles causantes de su efecto tipo antidepresivo.

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Aceite de hígado de tiburón del litoral norte de Cuba como ingrediente activo en el desarrollo de formulaciones farmacéuticas

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Introducción

El aceite de hígado de tiburón tiene entre sus principales componentes: escualeno, alquilglicerol, ácidos grasos omega-3, ácidos grasos libres, hierro, zinc y cobre. También es fuente de vitaminas liposolubles como A, D y E¹⁻³. Desde hace más de 40 años el aceite obtenido a partir de los tiburones ha sido utilizado como agente preventivo y terapéutico⁴. Hasta el presente, no existen antecedentes sobre la determinación de las especificaciones de calidad de la mezcla de aceite de hígado de tiburones costeros de Cuba.

Método

Para el aceite se desarrollaron y validaron métodos analíticos cromatográficos, para la cuantificación del contenido de vitaminas y ácidos grasos presentes en el mismo; las especificaciones de calidad se calcularon por el método de sustitución del valor de K tabulado; el estudio de estabilidad se realizó por 6 meses a una temperatura de 2-8 °C. Se realizaron dos procesos de microencapsulación, mediante secado por aspersión, con dos sistemas encapsulantes diferentes, a escala de laboratorio e industrial. Posteriormente se desarrolló una formulación de cápsulas duras empleando el aceite microencapsulado.

Resultados/Discusión/Conclusión

Se validaron los métodos analíticos para el aceite con resultados satisfactorios; se establecieron por primera vez las especificaciones de calidad y se demostró su estabilidad durante seis meses. En el proceso de microencapsulación del aceite, se establecieron los mejores porcentajes de agentes encapsulantes. Se

demostró que el proceso de microencapsulación fue efectivo factible y consistente a nivel. Se desarrolló una formulación de cápsulas duras empleando el aceite microencapsulado, como sustancia activa derivada de la mezcla de aceite de hígados de tiburones costeros de Cuba, con adecuada calidad tecnológica y estabilidad física, química y microbiológica durante 24 meses, a 30 ± 2 °C, 70 ± 5 % de humedad relativa. Se demostró que el aceite de hígado de tiburón se puede emplear como ingrediente activo en el desarrollo de formulaciones farmacéuticas.

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Study of different technological flows obtaining oil Moringa oil by cold president

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ECTI Sierra Maestra Project *Moringa*.

Summary

Moringaoleífera is a native plant of India that has a high nutritional value and numerous medicinal benefits. *Moringa* oil is very stable to oxidation, it also has healing and moisturizing properties for cosmetics, either in moisturizers, lotions, shampoos and ointments, among others. Through the process of cold extraction of oil, a purer oil is obtained and that does not lose its properties over time, in addition to having other uses, its greatest benefit is due to the power it presents for the skin. The objective of the work was to evaluate the most suitable productive flow to obtain a better oil yield. Seeds from India stored for 3 years were used for the study, which were cold pressed evaluating four different technological flows with three repetitions each. The study showed that there were no appreciable differences in terms of performance percentage in the 4 flows studied. It was determined that flow 1 was the most suitable since it consumed less time and less manipulation.

Determinación simultánea de vitaminas A y D, por CLAR/DAD, en el aceite de hígado de tiburón

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Introducción

Al aceite de hígado de tiburón se le atribuyen múltiples beneficios para la salud. Estudios científicos realizados han demostrado la presencia de vitaminas A y D, en el aceite de hígado de tiburón¹⁻³. Hasta el presente, no existen antecedentes sobre la determinación simultánea de vitaminas A y D, por CLAR/DAD, en la mezcla de aceite de hígado de tiburones costeros de Cuba.

Método

Para la cuantificación del contenido de vitamina A y D se empleó la Cromatografía líquida de alta resolución, con un detector de arreglo de diodo, a longitudes de onda de 210, 254, 280 y 340 nm. Se empleó un método por gradiente, con una fase móvil, agua destilada y ácido fórmico al 0,1%; acetonitrilo y ácido fórmico al 0,1 %. La separación se realizó a través de una columna RP-18 (Grace Smart), con un flujo de 1,0 mL/min y un dosificador de 20 µL. En el proceso de validación se evaluaron los parámetros de especificidad, linealidad, exactitud y precisión (repetibilidad y precisión intermedia).

Resultados/Discusión/Conclusión

El método analítico desarrollado por CLAR/DAD, resultó lineal, preciso, específico y exacto. La vitamina A es el componente mayoritario con relación a la vitamina D. Se demostró la aplicabilidad del método para el control de la calidad de la mezcla de aceite de hígado de tiburón del litoral norte de Cuba

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Formulation and evaluation of infusions of leaves of *Moringa oleifera* (moringa) and *Hibiscus sabdariffa* (flor de jamaica)

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Introduction

Moringa oleifera Lam (moringa) is a tropical plant used for medicinal and nutritional purposes. The interest of this plant as a functional food and as a source of nutraceuticals, is widespread worldwide, with the leaves and seeds being the most widely used due to the content of bioactive compounds (Leone et al, 2015).

Of *Hibiscus sabdariffa* L. (flower of Jamaica) most used is the calyx, due to its pharmaceutical and nutritional potential. Given the existing scientific evidence, the objective of this work was to design a formulation to prepare as an infusion, combining the moringa leaves with the chalice of the flower of Jamaica (Ali et al, 2005).

Methods

Raw materials were evaluated through residual moisture; total and acid insoluble ashes; protein and minerals. Aqueous extracts were prepared, to which the total phenol content was analyzed, by the Folin-Ciocalteu method; the antioxidant capacity (IC₅₀), by means of the DPPH test and by high efficiency liquid chromatography / mass spectrometry (CLAE / MS), the majority compounds were established.

For the formulation design a completely random combination was performed, evaluating mixtures of moringa and Jamaica in proportions of 50:50, 70:30 and 80:20, respectively. The most widely accepted formulation was packed in sealable filter paper bags, in an automatic packaging machine for tea bags without envelopes. For the quality control of the infusion were determined: refractive index, Bx, fructose (%), glucose (%), invert sugar (%), pH and relative density. In addition, the percent of total phenols was quantified for each 200 mL of the infusion and its corresponding IC₅₀.

Results



The quality standards determined for dry drugs are within the limits referred to in the literature. The quantification of phenols showed that the aqueous extract of Jamaica has a higher content of such metabolites (5,1388 g GAE/ 100g of raw drug) than a similar extract of moringa leaves (2,7483 g GAE / 100g of raw drug); which is directly related to the free radical sequestration capacity. The chemical study allowed to identify in the aqueous extract of moringa, glycoside flavonoids derived from quercetin, apigenin and kaempferol, phenolic acids and a lignan. In addition, organic acids and the amino acid phenylalanine. In the aqueous extract of the calyces of *H. sabdariffa* anthocyanins, glycoside flavonoids and organic acids were identified.

The most sensory acceptance formulation was the 50:50 mixture of both dry drugs. The physicochemical tests performed to the infusion facilitated its standardization. The quantification of polyphenols in 200 mL of it was 42.89 mg GAE and the DPPH inhibitory capacity was an IC₅₀ of 0.06 mg / mL.

Conclusions

The designed infusion has the characteristics necessary to be considered, for its nutritional and antioxidant properties, as a beverage beneficial to human health.

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Pharmacological evaluation of the aqueous extract of *Alternanthera sessilis* (L). r. br. in experimental psoriasis.

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Alternanthera sessilis (L). R. Br is a creeping plant or hairy grass of the *Amarantáceas* family, that usually is in the coasts and sandy grounds in Cuba and other latitudes; which is traditionally used for gastrointestinal, as well as astringent, abortive and diuretic activities. The study for the treatment of psoriasis was started taking into account the reference of its traditional use for urticaria. A fractionation procedure is carried out for the extraction and characterization of secondary metabolites of the plant; reporting the presence of carotenoids. A phytochemical study was carried out that included the determination of some pharmacognostic parameters, complying with the specifications established in standards and pharmacopoeias. For the identification of the secondary metabolites, phytochemical screening was performed on the extracts of the fresh plant and dried in an oven at 40 ° C, using the maceration method, identifying tannins, alkaloids, flavonoids, saponins and reducing sugars, these extracts were made with solvents of increasing polarity (ethyl ether, ethanol and distilled water). The pharmacological study to demonstrate the anti-Psoriasis effect of *Alternanthera Sessilis* L. R. Br Extract, carried out at the Center for Studies on Biological Evaluations and Research of the Institute of Pharmacy and Food of the University of Havana. The study was developed according to the technical requirements established in the Quality Assurance Plan of the institution. The results allowed to demonstrate the anti-psoriático effect of the extract in the murine experimental model used, verifying a regenerative effect of the epidermis; Coriodermin is used as a positive control. The novelty of this study is based on the finding of a new natural product that could be used as a therapeutic alternative for the treatment of psoriasis (Patent Certificate No. 24276 Resolution No. 2162/2017, Cuban Office of Industrial Property). In this sense, it refers to the pharmacological efficacy found for the aqueous extract of the plant *Alternanthera sessilis* (L). R. Br in the experimental model of Psoriasis studied is based on the active principles or secondary metabolites that have, referred to above, those that confer high antioxidant capacity, nutraceutical effects, which together with the trace elements that have been detrimental (Manganese, iron, Zinc, Magnesium, and Cobre among others) enhance their anti-psoriatic activity, activate the metabolism and the immune system, converting this plant and the extracts obtained from it into potentially active products for the treatment of various diseases, among which are Psoriasis.



Cuban endemic *Tabebuia* sp. as source of development of antitumor and antiinflammatory drugs

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Introduction

Cuban have more than 300 endemic *Tabebuia* species without any study by its use as medicinal. We made a pharmacological screening of the potentiality of three species in these genera as cytotoxic, cytostatic and anti-inflammatory.

Method

We probe the in vitro and in vivo anti- proliferative effect from the more promissory specie with different tumor cells and anti-inflammatory effect on acute a chronic inflammation tests. Chromatography techniques and GC/Mass spectrometry, NMR and Mass Spectrum were applicate to isolate and identified active compounds from promissory fractions.

Results/Conclusions

Some fractions from different organ of these plants was positive in pharmacological screening. *Tabebuia spp.* titled THH was the most cytotoxic and cytostatic and it shown anti proliferative in vitro an in vivo. However, fractions from TA and TO were active too. THH was different triterpenoid compounds with high quantity of compound 1. This compound shown antitumor effect and it had apoptosis activity. THH had anti-inflammatory activity. In conclusion, THH was a promissory candidate to development a new antitumor and anti- inflammatory drugs by human and animal health.





Estabilidad físico-química, química y microbiológica de extractos de hojas de *Azadirachta indica* A.Juss.

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Introducción

En la actualidad la tercera parte de los medicamentos sintéticos son elaborados a partir de plantas medicinales y el parasitismo intestinal no escapa a la acción de la medicina verde, ya que constituye un problema de salud a escala mundial¹, sobre todo en países tropicales y subtropicales, donde las infecciones por protozoos alcanzan una alta prevalencia. En Santiago de Cuba se conoce del uso popular de las hojas del árbol del Nim² (*Azadirachta indica* A.Juss) para tratar esta afección sin un basamento científico. En esta investigación, se evaluó la estabilidad de extractos de hojas de esta especie, que en un futuro podrían utilizarse en el diseño y elaboración de medicamentos a partir de la misma.

Método

Se evaluó la estabilidad físico-química, química y microbiológica de extractos de las hojas de *Azadirachta indica* A. Juss. , a tiempos 0, 7, 15, 30, 45 y 60 días a temperatura ambiente y en envase de cristal ámbar, a través de la determinación de parámetros de control de la calidad como características organolépticas, sólidos totales, densidad relativa, pH y cenizas totales. Los extractos de las hojas secadas a la sombra fueron elaborados por el método de percolación al 30% y 70%, y por decocción el acuoso. La evaluación se realizó a través de un análisis estadístico utilizando el programa StatGraphics CENTURION XV esp-eng para Windows del año 2005, realizando un Análisis de Varianza Simple, para evaluar si existían semejanzas o diferencias estadísticamente significativas entre los extractos.

Resultados/Discusión/Conclusión

El extracto acuoso no fue estable. El extracto hidroalcohólico al 70% en base a los parámetros analizados no varían durante el tiempo de la investigación, lo que nos demuestra, que la estabilidad del mismo no se ve afectada ni por la influencia de las condiciones de almacenamiento, ni envase. El extracto hidroalcohólico al 30%, mantuvo un comportamiento inestable, en los parámetros físico-químicos evaluados durante el tiempo de estudio. El extracto más estable fue el elaborado a partir de alcohol al 70%.

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Self-emulsifying drug delivery systems, seddsof plant extracts with bioactive potential (*Malus domestica*, *Prunus domestica*, *Psidium guajava*)

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Introduction

Plant phenolic compounds are substances that include at least one phenolic group in their chemical structure. Polyphenols are characterized by having high antioxidant potential, which can be beneficial in the prevention and treatment of many human diseases related to the accumulation of oxidative stress, including cancer and cardiovascular and neurodegenerative problems, diabetes, autoimmune diseases, among others [1-5]. An advantage of these substances is that they are part of many foods (fruits, vegetables, spices, tubers, grains, cereals, seeds, drinks, etc.). Therefore, they are substances that are normally part of the human diet, which means that they can be consumed orally, which facilitates their administration. The first limiting step that affects the bioavailability of the compounds is their absorption in the gastrointestinal tract. Most of the absorption occurs in the small intestine, where the enterocytes are responsible for transporting nutrients (sugars, amino acids, fats, vitamins, minerals) from the intestinal lumen into the bloodstream. Polyphenols and many other phytochemicals are not nutrients, they are not essential substances for our body (they are xenobiotics). Therefore, some phytochemicals can not be absorbed: because they are not permeable to the intestinal barrier, because they are very large, or because there is no adequate transport mechanism. In addition, once inside the enterocytes, most phytochemicals are metabolized; that is, they are chemically transformed to improve their circulation in the blood and facilitate their rapid excretion in the urine. These modifications usually improve the aqueous solubility of the substances, but change their chemical composition, which can reduce or even eliminate their bioactive potential. Micelles and microemulsions are aggregates of amphipathic molecules, molecules that have a hydrophilic end and another hydrophobic end. When these molecules are mixed in water, the hydrophobic ends tend to bind to each other, because this part is repelled by the polarity of the water. When the amphipathic molecules reach the "critical micellar concentration", aggregates form spontaneously, which have the shape of small spheres. When the micelles self-assemble, they can trap other molecules that have been present in the solvent during the preparation, and that have greater affinity for the interior of the micelle than for the original solvent. This is especially useful for encapsulating hydrophobic substances, which are also those that normally have low bioavailability in vivo, since they tend to precipitate in the gastrointestinal tract, forming large aggregates that can not be absorbed and end up being excreted in the feces without having arrived never to the blood circulation. For this study, micelles based on polysorbates, specifically Tween 20 and Tween 80, will be prepared, which are amphipathic substances widely used in the food industry and considered edible additives [23]. The micelles



will be used to solubilize ethanolic extracts of fruit (peel and pulp) of apple (*Malus domestica*, variety Ana), plum (*Prunus domestica*, variety satsuma) and guava (*Psidium guajaba*).

Method

The characterization of total polyphenols (equivalents of gallic acid) and some specific polyphenols (catechin, epicatechin, quercetin, ferulic acid and / or cyanidin-3 glucoside) and total carotenoids will be carried out using HPLC methods. A method of general detection of polyphenols (using catechin and quercetin as a standard) will also be established and used by HPLC. In addition, they will perform analysis of brix degrees, humidity and acidity. The physicochemical characteristics of each extract will be shown in a descriptive table when publishing the results of each species, and could be purchased between species. The antioxidant activity of the powder extracts and of the extracts solubilized in micelles of each species was characterized, using the ORAC assay. The cytotoxic activity of the powder extracts and the solubilized extracts will be characterized in at least 5 concentrations for each extract (based on a reference compound) against cancer cells of skin, liver and colon, using the neutral red test. Three replications of three replicates will be made for each experiment.

Results/Discussion/Conclusion

Preliminary tests of extraction of polyphenols were carried out with apples of the varieties Gala ($n = 6$) and Ana ($n = 6$) purchased at the supermarket. Two drying methods (dehydration and lyophilization) were compared and the dried material was extracted once by maceration (1:10 or 1:20, fresh material: solvent) under constant stirring (> 4 h) and sonication (30-40 min) with different proportions of ethanol (50, 70 and 95%) in distilled water. On average, ca. 690 mg total polyphenols (mg equivalents of ellagic acid, Folin-Ciocalteu method) of lyophilized material ($n = 9$) and ca. 380 mg total polyphenols of dehydrated material (from a single sample), showing little difference between solvents and numerically higher concentration of total polyphenols in fruits var. Ana. The method of best recovery (lyophilization and maceration with 50% ethanol) will be repeated to compare between apples (var. Ana) at different stages of maturation. The extracts with the highest content of total polyphenols will be nanoencapsulated in micelles edible surfactants.

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Comparative Study of Essential Oils Extracted from *Plectranthus Amboinicus* (Lour.) Spreng. Leaves Using Microwaves and Hydrodistillation

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Introduction

Essential oils (EOs) are complex mixtures of volatile organic compounds produced in the form of secondary metabolites in plants that can contain about 20-60 components in very different concentrations [1-7]. They are characterized because they contain two or three main components in relatively high concentrations



(20-70%) compared to other components present in minimal amounts [8-10]. They may consist of monoterpenes, sesquiterpenes, and phenylpropanes, which may contain different functional groups (alkanes, alcohols, aldehydes, ketones, esters and acids) [11,12].

In nature, EOs plays an important role in the protection of plants such as antibacterials, antivirals, antifungals, insecticides and also against herbivores, reducing their appetite for these plants. They are also responsible for the characteristic smell of plants, which can attract some insects to favor the dispersal of pollen and seeds, or repel other undesirable ones [13,14]. Around 3000 of the essential oils are known and 10% of them having commercial importance in the cosmetic, food and pharmaceutical industries, and in agriculture [12,15]. Therefore, they are generally recognized as safe by the FDA (Food and Drug Administration). Its composition can vary considerably between species of aromatic plants and varieties and within the same variety of different geographical areas [12].

The main methods to obtain essential oils from the plant materials are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic (or destructive) distillation, and expression [16]. Among these methods, HD has been the most common approach to extract the essential oils from the medicinal herbs/plants [6,8]. However, in order to reduce the extraction time and possibly improve the extraction yield, to enhance the quality of the extracts and also to reduce the operation costs, new approaches such as microwave-assisted extraction (MWHD), pressurized solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought [16,17].

Plectranthus amboinicus (Lour.) Spreng. is a tender fleshy perennial plant in the family Lamiaceae with oregano like flavor and odor, it has deltoid-shaped, crenate leaves that are hard and brittle. The species has a wide geographical distribution, and is found in central Africa, Asia, and South America. Reported for many traditional uses, especially for the treatment of cough, sore throat and other nasal congestion [12]. It is also used for a range of other problems such as infection, rheumatism and flatulence [1-4,12]. Therefore, the objectives of this study were to investigate the potential of MWHD for the extraction of essential oils from dried *P. amboinicus* L. An attempt has also been made to compare extraction time, extraction yield/efficiency and aromatic composition of the extracts with those of HD.

Method

2.1 Collection of plant material. Fresh leaves of *P. amboinicus* L. were collected in two different places: in Pamplona (Norte de Santander) and Cartagena (Bolívar), in Colombia. The plant material was certified by top experts from Catatumbo Sarane Regional Herbarium (HECASA) of the University of Pamplona, Norte de Santander, Colombia. Certified species was then kept in a dark and cold room until used shortly after that for the experiments.

2.2 Extraction of essential oils. The EO was obtained by two methods: hydrodistillation steam with conventional water and microwave assisted hydrodistillation. For that, it was used a hydrodistillation equipment with 4 liters of capacity. 500 g of plant material were taken, then they were introduced into the extraction flask, which contained 500 mL of distilled water. In both the steam process where a heating mantle was used as in the microwave assisted, the extraction time was 3-4 hours. As a source of microwave radiation, a conventional modified oven brand Samsung was used, with a 1-cycle irradiation of 60 minutes and a power of 70% [6,16,17]. In both cases the EO was collected in a DeanStark type vessel.

2.3 Physical constants. Specific gravity, refractive index of the essential oils extracted from the *P. amboinicus* samples (by both methods) were measured according to the method suggested by Food Chemical Codex. Specific gravity was measured at 25 C. Refractive index was measured at 20°C [6,16].

2.4 Determination of the major components of EO by gas chromatography-mass spectrometry (GC / MS). An Agilent 7890A / 5975C chromatograph was used. Each EO sample (50 µL) was dissolved in 450 µL dichloromethane, the injector temperature was 250°C, a HP-5MS 5% Phenyl Methyl Silox capillary column was used; Helium was used as carrier gas at constant flow rate of 1 mL / min, pressure of 7.6354 psi and linear velocity of 36 cm / sec. Initial temperature 45°C and transfer line temperature 280°C. Mass spectra were obtained by electron ionization (70 eV), with automatic scanning at a range of m/z 30-400 u.m., at 3.85 scan / s. The components identities were assigned by comparison of each spectrum with the database standards reported in the literature [6,18].

2.5 Cytotoxicity assay

According to the literature, an *in vitro* test was used for the evaluation of essential oils in HepG2 cells. The methodology used was based on *in vitro* tests with HepG2 cells, evaluating the toxicity of essential oils [9].

Briefly, HepG-2 cells (from human liver, hepatocellular carcinoma; American Type Culture Collection (ATCC) HB8065) were seeded into 96-well plates at 1×10^4 cells per well and incubated for 24 h at 37° C. The medium (DMEM/F12) was removed and fresh medium containing compound at concentrations of 0.01, 0.1, 1, 10 and 100 $\mu\text{g}/\text{mL}$ was added for 72 h at 37° C. After that, medium was removed and MTT solution (20 μL , 5 mg/mL, Sigma Co.) was added to each well. The extent of MTT reduction to formazan was quantified by measuring the optical density (OD) at 580 nm, the percentage of mortality was determined [9].

2.6 Test microorganisms

The microorganisms used in the study were obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

2.6.1 Minimum inhibitory Concentration (MIC)

The MIC was determined of those essential oils that showed inhibition percentages higher than 90%, following the established by the CLSI with some modifications. Briefly, 50 μL of the suspensions of bacterial strains in study were incubated, for the period of time defined for each bacterium in the bacterial growth curves, at 35 ± 2 °C, in 96 wells plates, with 50 μL of serial concentrations between 50 and 1000 $\mu\text{g}/\text{mL}$ of the evaluated extract. The plates were sealed during the incubation period to reduce evaporation; At the end of this time, they were agitated at 100 rpm for 5 min and the DO_{620} was determined in microplate reader. The MIC was calculated as the minimum concentration of the extract or fraction that completely inhibits bacterial growth and was expressed in $\mu\text{g}/\text{mL}$. [15].

2.6.2 Minimum Bactericidal Concentrations (MBC)

To determine the concentration of the essential oil that showed complete inhibition of bacterial growth, in the test of determination of the MIC, an inoculum was taken with a sterile handle and a subculture was made in Petri Dishes with MH agar. The inoculated plates were incubated at 35 ± 2 °C during the appropriate time for each bacterium, after which it was evaluated if there was growth of bacterial colonies. The MBC was calculated as the minimum concentration of the compound that does not allow visible growth of colonies on the Petri dish. If growth is observed, it is concluded that this concentration of the extract or fraction produces a bacteriostatic effect [15,19,20].

2.7 Methods to determine the radical scavenging ability and measurement of the antioxidant activity of EO. Two methodologies were used to determine the *the radical scavenging ability* DPPH (2,2-diphenyl-1-picrylhydrazole) and ABTS (2,2'-azino-bis- (3-ethylthiazolin-benzenesulfonic acid-6)). The antioxidant capacity was measured by ORAC method.

2.7.1 Radical method DPPH•. Free radical scavenging activity DPPH was determined using the method described by Silva et al. [21] (with some modifications 75 μ L of sample were added to 150 μ L of a methanol solution of DPPH (100 ppm) and they were incubated at room temperature for 30 min, after the disappearance of the DPPH radical was determined spectrophotometrically at 405 nm in microplate reader Multiskan Ex (Thermoscientific). Ascorbic acid (25 μ g / mL) was used as a positive control.

$$\% \text{ Inhibition} = \frac{(A_0 - A_f)}{A_0} * 100$$

Where A_0 and A_f are the absorbance values of blank (DPPH solution in alcohol) and the sample (DPPH solution plus alcohol-dissolved), respectively.

2.7.2 Radical method ABTS^{•+}. The free radical scavenging activity ABTS was determined using the method described by Re *et al.* [22] with some modifications. The ABTS radical was formed following the reaction of 3.5 mM ABTS with 1.25 mM of potassium persulfate (final concentration). The samples were incubated at 5°C and in darkness for 16 h. Once the ABTS radical was formed, it was diluted with ethanol until having an absorbance of 0.7 ± 0.05 at 734 nm. To a volume of 190 μ L of the ABTS radical dilution was added 10 μ L of the EO sample and incubated at room temperature for 5 minutes. After this time, the disappearance of the ABTS radical at 734 nm was determined spectrophotometrically in the microplate reader Multiskan Ex (Thermoscientific). Ascorbic acid (4 μ g / mL) was used as a positive control for the uptake of ABTS radicals.

2.7.3 ORAC Method (hydrophilic and lipophilic). Hydrophilic ORAC: trolox (TX) was used as standard and controlled conditions of temperature (37°C) and pH (7.4) were used in this evaluation. The readings were performed at an excitation de of 493 nm and emission of 515 nm. For the technique development, they were used 1×10^{-2} M fluorescein solutions in PBS (75 mM) and 0.6 M AAPH (2,2'-Azobis(2-amidinopropane)





dihydrochloride) in PBS (75 mM). The sample was prepared by mixing 21 μ l fluorescein, 2,899 μ l PBS, 30 μ l extract (test sample) and 50 μ l AAPH. Trolox was used as reference. The protective effect of the antioxidant was calculated using the area differences under the decrease of the curve of fluorescein with blank and the sample, and the result was compared with curve obtained with trolox. The results were expressed in micromoles of trolox equivalents per 100 grams of sample (μ mol Tx / 100 g sample), according to the following equation.

$$ORAC = \frac{(AUC - AUC^{\circ})}{(AUC_{Trolox} - AUC^{\circ})} f [Trolox]$$

Where AUC is the area under the curve of the sample, AUC° is the area under the curve of the control, AUC_{Trolox} is the area under the curve of trolox, f is the dilution factor of the extracts.

Lipophilic ORAC: it was follow the previous described procedure except that the sample and trolox were prepared with methylated cyclodextrin (7%) and in a 50% acetone-water mixture. The solutions were shaken for 1 hour and the analysis was performed as described in the hydrophilic ORAC method [7,23,24].

2.8 Statistic analysis. All trials were performed by sextupled. The results were expressed as the mean \pm SD (standard deviation). Significant differences were determined by ANOVA analysis followed by Dunnett's or Tukey's test, or as appropriate.

Results / Discussion / Conclusion

Figure 1 shows the extraction kinetics of the obtained EO, after 40 minutes of extraction of EO by MWHD resulted a recovery similar to those obtained for 3 hours of HD. Reaching final yields by MWHD and HD method of 0.17% and 0.10% from Cartagena plants and 0.23% and 0.15% from Pamplona plants respectively. These results indicate that the MWHD technique uses less extraction time, this because of the breaking of the structures of the main components of greater abundance in the EO by electromagnetic radiations applied in the extraction; the microwaves involve a more efficient heat flow, and they can heat the entire sample almost simultaneously at a high rate, generating higher performance and lower power consumption in the MWHD method compared to HD. Microwave assisted hydrodistillation uses three forms of heat transfer within the sample: irradiation, conduction, and convection. As a result, it produces heat more quickly inside and outside the glands. With HD, this heat transfer can only occur through conduction and convection, which makes it less effective [6,16].



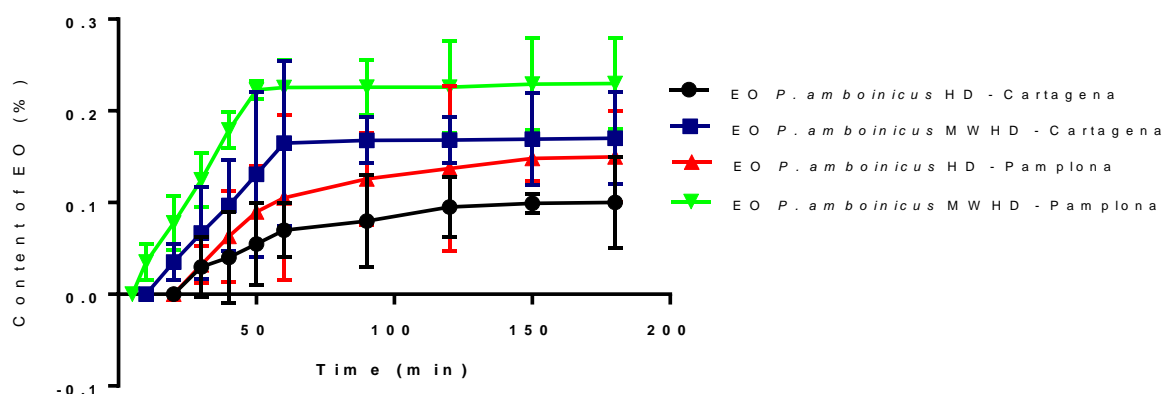


Figure 1: Kinetic extraction of essential oils (EO) obtained by the method of steam distillation (HD) and microwave assisted hydrodistillation (MWHD)

Essential oil yield from specimens of *Plectranthus* may vary between 0.1 and 0.3%, and our findings were in this range, where 0.10% (HD) - 0.17% (MWHD) oil was obtained from Cartagena plants and 0.15% (HD) - 0.23% (MWHD) from Pamplona plants, which can be explained by local differences in soil, climate and relative humidity. This differed from other studies [19] which quantified the essential oil of four species of *Plectranthus* and found that *P. amboinicus* L. had the highest essential oil content with 0.43%. The difference in oil constituents found between the two samples could have been due to the environmental characteristics of the sampling sites. Cartagena is located in a region with higher temperature and higher relative humidity. Climate issues might have also played a part in essential oil composition [12].

Physical properties (appearance, refractive index and specific gravity) of *P. amboinicus* essential oils extracted by both HD and MAHD are shown in Table 1. The refractive indices and specific gravities of essential oils obtained from thyme. The only difference was that the color of the essential oils extracted by MWHD was lighter than that obtained by HD. Therefore, considering physical properties of the extracted essential oils, MWHD as a new extraction technique does not introduce any problems to the essential oils extracted from *P. amboinicus*.

TABLE 1: Physical properties of essential oils from *P. amboinicus* obtained by hydrodistillation (HD) and microwacce assisted hydrodistillation (MWHD)

Physical properties	<i>P. amboinicus</i> (Cartagena)		<i>P. amboinicus</i> (Pamplona)	
	HD	MWHD	HD	MWHD
Specific gravity (25 °C)	0.947	0.952	0.948	0.955
Refractive index (20 °C)	1.4755	1.4755	1.5078	1.5078
Appearance	Yellow	Pale yellow	Yellow	Pale yellow

Table 2 presents the majority components present in the EO obtained by the steam distillation hydrodistillation and microwave assisted hydrodistillation methods.

TABLE 2: Major components detected in EO obtained by HD and MWHD

Component	% Relative abundance, (t _R , min)			
	<i>P. amboinicus</i> (Cartagena)		<i>P. amboinicus</i> (Pamplona)	
	HD	MWHD	HD	MWHD
γ-Terpinene	6.20 (13.336)	7.12 (13.898)	6.67 (13.358)	8.22 (13.223)
Thymol	13.39 (14.855)	15.40 (14.859)	14.78 (14.952)	15.95 (14.855)
Carvacrol	64.55 (19.055)	69.07 (19.003)	66.11 (19.001)	70.09 (18.901)
Caryophyllene	4.46 (21.211)	5.23 (21.098)	5.07 (21.080)	5.54 (21.286)

Retention time (t_R) and relative abundance (%) of the essential oils, identified by comparison with the reference mass spectrum of the NIST-2008 database.

P. amboinicus L. is rich in essential oils. The chemical composition of the essential oil of *P. amboinicus* L. was determined by gas chromatography coupled to mass spectrometry (GC/MS), and as a result the components were identified by comparing their mass spectra and retention times (RT) with those in the literature [12]. In the table 2 are listed all phytocompounds identified on both essential oils. In the sample collected in Pamplona, 92.63% (HD) – 99.80% (MWHD) of the constituents were identified. In the sample collected in Cartagena and analyzed by the same process, 88.60% (HD) – 96.82% (MWHD) of the constituents were identified. In both essentials oils the majority components was Carvacrol, followed by Thymol, γ -Terpinene and Caryophyllene.

Ranasinghe *et al.* [5], demonstrated that eugenol, carvacrol, thymol and cinnamaldehyde are the main components of EO, which provide antifungal and antioxidant activity. It is important to note that thyme EO obtained by the two extraction methods have the thymol as the major component, and it also presents, to a lesser extent, γ -terpinene, which is a cyclic monoterpene biosynthetic precursor of thymol and carvacrol. Castillo *et al.*, [14] found that EO thyme antioxidant activity depends on the content of thymol and carvacrol. It should be noted that MWHD method is an excellent alternative because it is a green, eco-friendly compared to other solvent extraction techniques such as Soxhlet, since it avoids the use of organic solvents [6,16,17].

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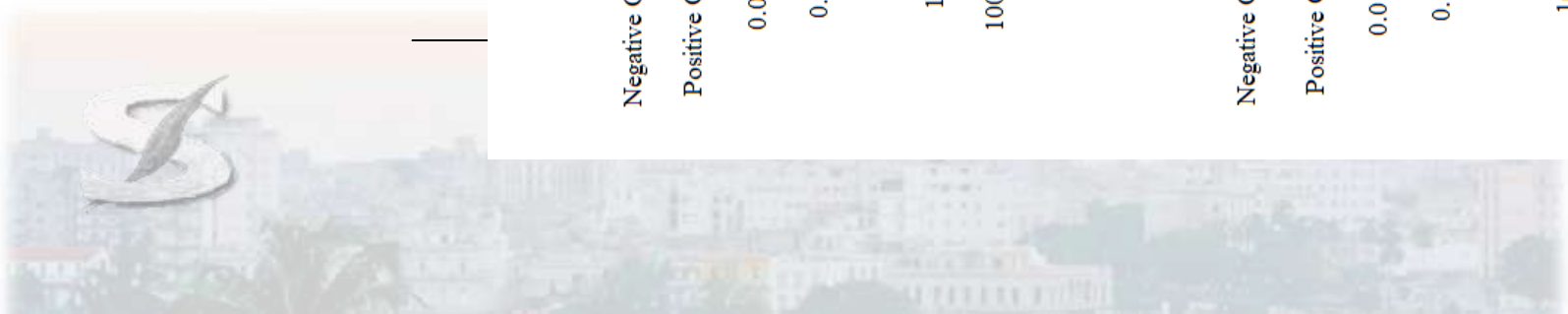
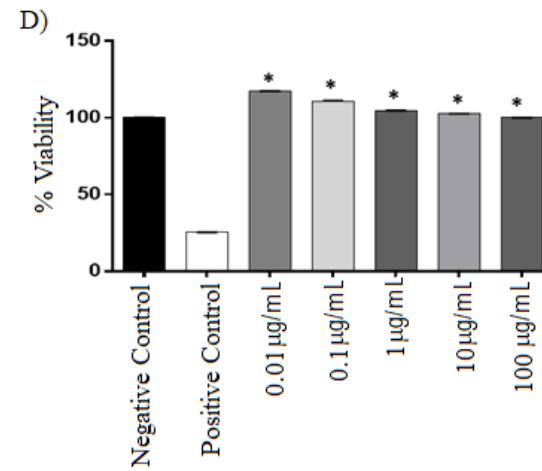
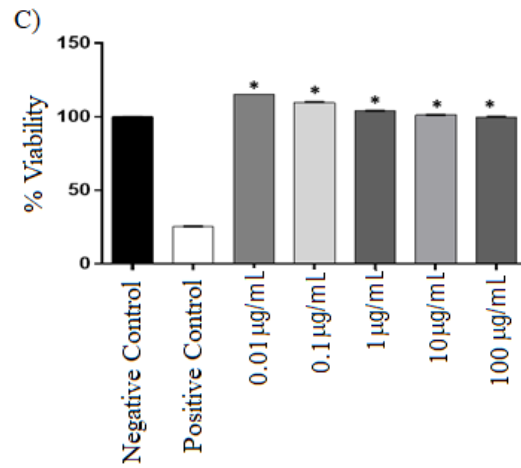
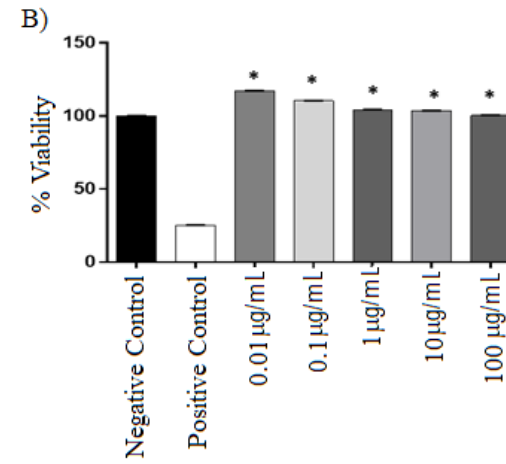
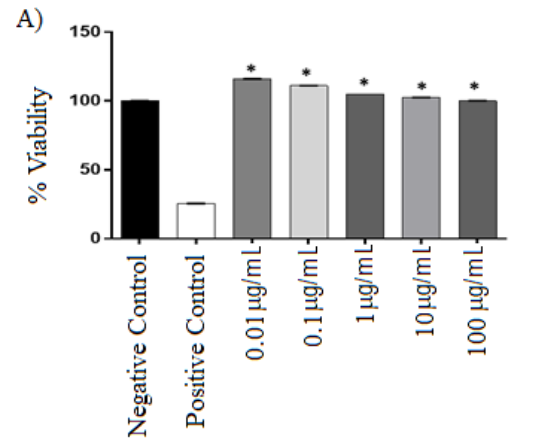


Figure 2: Results of MTT assay for HepG2 cells exposed to a) Essential oil of *P.Amboinicus* (Cartagena) essential oil extracted by HD; b) Essential oil of *P. Amboinicus* (Cartagena) extracted by MWHD; c) Essential oil of *P.Amboinicus* (Pamplona) essential oil extracted by HD; d) Essential oil of *P. Amboinicus* (Pamplona) extracted by MWHD;* Significaticas Differences compared to control, $p > 0.05$, One-Anova, Dunnett's test

Over the years, a number of techniques have been reported for the determination of in vitro cytotoxicity and cell viability, being sensitive and reliable low-cost techniques. Also, you have been applied to investigate plant cytotoxicity profiles and chemotherapeutic agents [9].

Cell cytotoxicity tests are widely used in in vitro toxicology studies. The MTT Assay is one of the most used methods for the study of cell cytotoxicity or viability followed by exposure to toxic substances [9].

According to the foregoing, the results of the MTT assay in this study after exposure of the HepG2 cells to the different concentrations of the essential oils are presented in Figure 2. The results show that there were statistically significant differences ($p < 0.05$) between the viability values of the positive control in relation to the different concentrations of the different essential oils by the two extraction methodologies evaluated in the trial, making it clearly a wide-ranging therapeutic component. As in evaluating the controls, the positive control showed a significant decrease in cell viability. This is in accordance with the data presented in the literature [9].

The growth tests revealed that the strains of *S. aureus*, *S. Epidermidis*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* reached the highest DO_{620} at 20 h; therefore, these were the times of incubation end point in the bioassays of antibacterial activity. To dissolve the essential oils evaluated in DMSO at 1%, first the bacterial growth was evaluated and it was shown that this solvent did not inhibit the viability of any of the strains; therefore, it was decided to use a broth system: DMSO in proportion 99:1. The results of the evaluation of the antibacterial sensitivity of the essential oils obtained by the method of distillation by steam trawl (HD) and microwave-assisted hydrodistillation (MWHD), allowed to identify the extracts able to inhibit the growth of bacterial strains, taking as selection criterion, those who were able to inhibit in more than 90% the three strains (table 3).

TABLE 3: Antibacterial sensitivity of the essential oils of *P. Amboinicus* obtained by the distillation method (HD) and microwave-assisted hydrodistillation (MWHD).

Essential oils (EO)	Bacterial growth inhibition percentage				
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
<i>P. amboinicus</i> (Cartagena)HD*	90.90 ± 0.33	90.50 ± 0.50	92.80 ± 0.22	93.18 ± 0.11	92.50 ± 0.25
<i>P. amboinicus</i> (Cartagena)MWHD*	91.30 ± 0.20	92.09 ± 0.20	93.50 ± 0.12	94.11 ± 0.56	92.95 ± 0.27
<i>P. amboinicus</i> (Pamplona)HD*	91.50 ± 0.22	90.88 ± 0.80	92.90 ± 0.15	93.79 ± 0.12	92.70 ± 0.15
<i>P. amboinicus</i> (Pamplona)MWHD*	92.00 ± 0.50	92.35 ± 0.21	93.89 ± 0.10	94.59 ± 0.37	93.08 ± 0.11
Gentamicin (control)	98.10 ± 0.33	98.00 ± 0.50	97.00 ± 0.55	98.00 ± 0.25	98.00 ± 0.33

* Essential Oils that inhibited more than 90% of the five strains. The values correspond to the average of three independent tests ± standard deviation.

Therefore, the MIC and MBC were determined. The MIC is determined by the use of inoculated and standardized broth, which is added essential oil solutions at different concentrations, causing a dilution. The values are shown in table 4. For its part, the results of MBC for essential oils suggest that these concentrations their activity is due to effects of type bacteriostatic and non-bactericidal (table 4).

TABLE 4: Minimum inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of essential oils against *S. aureus*, *S. Epidermidis*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*.



Bacterial Strains	Essential oils (EO)	Method of extraction	MIC (mg/mL)	MBC (mg/mL)
<i>S. aureus</i>	<i>P. amboinicus</i> (Cartagena)	HD	900	> 1,000
		MWHD	700	> 1,000
	<i>P. amboinicus</i> (Pamplona)	HD	900	> 1,000
		MWHD	600	> 1,000
<i>S. epidermidis</i>	<i>P. amboinicus</i> (Cartagena)	HD	900	> 1,000
		MWHD	700	> 1,000
	<i>P. amboinicus</i> (Pamplona)	HD	900	> 1,000
		MWHD	600	> 1,000
<i>K. pneumoniae</i>	<i>P. amboinicus</i> (Cartagena)	HD	800	> 1,000
		MWHD	600	> 1,000
	<i>P. amboinicus</i> (Pamplona)	HD	700	> 1,000
		MWHD	600	> 1,000
<i>P. aeruginosa</i>	<i>P. amboinicus</i> (Cartagena)	HD	800	> 1,000
		MWHD	600	> 1,000
	<i>P. amboinicus</i> (Pamplona)	HD	800	> 1,000
		MWHD	600	> 1,000
<i>E. coli</i>	<i>P. amboinicus</i> (Cartagena)	HD	700	> 1,000
		MWHD	600	> 1,000
	<i>P. amboinicus</i>	HD	700	> 1,000

	(Pamplona)	MWHD	600	> 1,000
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It should be noted that the essential oils showed a greater inhibitory effect against Gram-negative strains. This particular behavior compared to a group of strains, probably because the cell wall of the Gram-positive bacteria studied is basically composed of peptidoglycan that represents up to 90% of the wall, teichoic acids that are also usually be present in small quantities and polysaccharides; While the cell wall of the Gram-negative is constituted only by 10% of the peptidoglycan, also has three polymers that are outside its sheath: lipoprotein, outer membrane and lipopolysaccharides, the latter with a lipid content that It could favour the entry, by dissolution of essential oils due to its hydrophobic character and cause cell death by destabilization of the external membrane and the plasma membrane. This lipid A is not present in Gram-positive bacteria and this would be a probable cause of the effect of oil on the different bacterial groups, where Gram-negative bacteria are relatively more sensitive [15].

The hydrophobic nature of EO allows it to traverse the cell wall of Gram-negative bacteria through channels composed of proteins called porins, which are found in the outer membrane and facilitate the transport of nutrients and substances of Low molecular weight within the cell, including Antimicrobial agents [15, 20]. The components of the essential oils could exert antibacterial activity by interfering in the bilayer of phospholipids of the cell membrane causing the increase of its permeability and loss of the cellular constituents, since it destroys the system of enzymes including those involving the production of cellular energy (proton motive force) and bacterial respiration, when it comes to low concentrations of essential oils; while high concentrations would cause severe damage to the structural components of the bacterial cell, such as loss of homeostasis or inactivating or destroying the genetic material, resulting in cell death. In the mechanism of action of essential oils as antibacterial agents, it should be considered the large number of chemical compounds that are present in these, whose antibacterial activities do not present a specific mechanism [15, 20].

Table 5 presents the results obtained in the determination of the antioxidant capacity of the samples from the AE extracted by the methods HD and MWHD analyzed by the methods DPPH[•], ABTS^{•+} and ORAC.

TABLE 5: Radical scavenging ability and antioxidant capacity of extracted EO by the HD and MWHD methods analyzed by the DPPH, ABTS and ORAC.

Essential oil	Extraction method	Methods to determine the radical scavenging ability and antioxidant activity			
		DPPH [*] IC ₅₀ (µg/mL)	ABTS ^{*+} IC ₅₀ (µg/mL)	ORAC mmol trolox/100 g sample	
				Hydrophilic	Lipophilic
<i>P. amboinicus</i> (Cartagena)	HD	265.8±1.15*	29.00±0.10*	395.0±0.25*	686.50±0.33*
	MWHD	210.5±0.50*	21.11±0.25*	415.5±0.33*	705.90±0.25*
<i>P. amboinicus</i> (Pamplona)	HD	290.5±0.35*	28.80±0.37*	403.2±0.45*	695.10±0.55*
	MWHD	200.4±0.22*	20.09±0.11*	429.2±0.25*	713.50±0.77*

* Significant differences, $p < 0.05$

The antioxidant activity of the essential oils of *P. Amboinicus*, were evaluated by three different methods: DPPH^{*}, ABTS^{*+} and ORAC. Antioxidants can act by multiple mechanisms, depending on the reaction system or the source of radicals or oxidant used. The results are expressed as antiradical activity or IC₅₀, which is defined as the concentration of antioxidant that decreases the absorption of the radical to 50% of the initial amount [24].

The IC₅₀ value measures the catching capacity of radicals DPPH^{*} or ABTS^{*+} of the OE with respect to an antioxidant standard as the ascorbic acid, which yields a hydrogen, producing a transfer of electrons of double bond, towards the oxygen, which suffered the loss of an electron, repeating the same action in the next oxygen atom, which suffered the loss of the hydrogen atom, until the equilibrium of energy was established. According to this reaction, ascorbic acid yields two hydrogens [23].

The results show that for the essential oils obtained the values of ABTS^{*+} expressed as µg/mL, are lower than those obtained with the technique of DPPH^{*}; because the discoloration method of the cation-radical

ABTS^{•+} is applicable to lipophilic and hydrophilic antioxidants which allows it to be implemented for both aqueous and lipophilic systems, in addition, the ABTS is very soluble in water and chemically stable, in change, the DPPH[•] can only be dissolved in organic environment, which preferentially measures the antioxidant capacity of little polar or non-polar compounds [7,23,24].

MWHD offered substantial advantages over conventional HD. A similar extraction yield was achieved at significantly shorter extraction time when using MWHD instead of HD. Therefore, considering the operation cost MWHD could be carried out using half of the expenses required by HD. GC-MS results indicated that there were no significant differences between the essential oils obtained by MWHD and those obtained by HD proposing MWHD as an excellent alternative for HD with no adverse effects on the composition of the extracted essential oils. Stressing that the process of microwave assisted hydrodistillation is considered a fast, efficient, green and relatively economical method compared to conventional hydrodistillation. Chemically OE contains mainly monoterpenes being the Carvacrol which is found with a higher percentage of relative abundance, stressing that the essential oils obtained by MWHD presented promising results; This makes this AE an excellent asset for the design of master products with antioxidant and antibacterial activity.

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Serious adverse reactions to phyto-drugs in primary health care. Cuba, 2007-2017

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Introduction

At present the phyto-drugs are widely consumed internationally, mainly in primary health care, but there are few studies that show their safety. The objective of the work is to characterize the serious adverse reactions to phyto-drugs in Primary Health Care, notified to the Central Pharmacovigilance Unit from 2007 to 2017 in Cuba.

Method

A descriptive, observational and transversal pharmacovigilance investigation was carried out. The universe consisted of 812 reports of suspicion of reactions and serious adverse reactions to phytopharmaceuticals registered in the national pharmacovigilance database from 2007 to 2017. The variables studied were sex, age, adverse drug reactions, organ system, phyto-drug, Notifier, imputability, severity and frequency. It used the descriptive statistic.

Results

Suspected adverse reactions to phytopharmaceuticals predominated in females with 544 (67.0%) and adults (67.4%). The digestive system was the most affected and epigastric pain, vomiting, dermatitis and hypotension were the most reported, prevailing in *Allium sativum* L with 103 (12.7%), *Cymbopogon citratus* (DC.) Staph with 87 (10.1%) and *Aloe vera* L. with 42 (5.2%). The doctors reported 272 (33.5%). 68.5% (556) were probable, 810 (99.7%) moderate, 245 (30.2%) frequent and 181 (22.3) not described.

Conclusion

The series studied allows describing a safety pattern with the use of phytopharmaceuticals in the Primary Health Care of the country. The moderate reactions were predominated in female adult patients. *Allium sativum* L was the most related phytopharmaceutical, affecting the digestive system.

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Potential chemopreventive and therapeutic effects of cynaropicrin a sesquiterpene lactone from *Centaurea drabifolia* sm. subsp. *detonsa* (BORMN) Wagenitz

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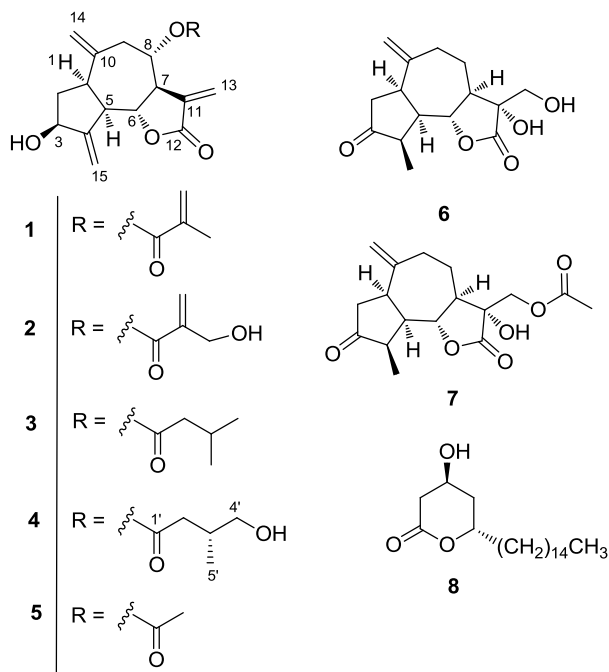
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Introduction

Centaurea genus belonging to the Asteraceae family is represented by approximately five hundred species distributed through the World. One hundred and ninety species are grown in Turkey, one hundred of which are endemic (Davis et al.; 1988). *Centaurea* species have been widely used for their antidandruff, antidiarrheic, antirheumatic, antiinflammatory, choleric, diuretic, digestive, stomachic, astringent, antipyretic, cytotoxic, and antibacterial properties in folk medicine. Previous phytochemical studies have revealed the presence of acetylenic compounds, flavonoids and sesquiterpene lactones (Formisano et al.); 2012. To our knowledge, no phytochemical investigation has been reported on *C. drabifolia* subsp. *detonsa* endemic in Turkey.

Results

The investigation on chloroformic extract of this plant led to the isolation of seven guaianolide sesquiterpene lactones, two flavonoids and one aliphatic lactone. All compounds were isolated for the first time in this species, and among them the guaianolide (**4**) is a new natural compound. The anticancer activities of the compounds against melanoma were determined. Results showed that cynaropicrin (**2**) showed a potent activity inhibited the proliferation of several melanoma cell lines (A375, SK-Mel 28, WM983B, WM3060) in time- and dose-dependent manner, revealing interesting details about the structure-activity relationships in the class of acylated guaiane sesquiterpenes.



Centaurea L. which is an important genus of Asteraceae (Compositae) family is distributed with its ca. 700 species in Asia, North Africa, America, and Europe.[1][2]*Centaurea* is represented in Turkey with 34 sections and 226 species with an endemism rate of 66%.[3] *Centaurea* species have been widely used for their antidandruff, antidiarrheic, antirheumatic, anti-inflammatory, choleric, diuretic, digestive, stomachic, astringent, antipyretic, cytotoxic, and antibacterial properties in folk medicine. [4][5]

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Análisis físico, químico y enantiomérico del aceite esencial de la planta *Lepechinia betonicifolia* (Lam.) Epling de la provincia de Loja.

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Introducción

La presente investigación describe el análisis del aceite esencial de la especie *Lepechinia betonicifolia* (Lam.) Epling recolectada en la provincia de Loja, para lo cual se realizó la extracción del aceite esencial a partir de las hojas de la planta mediante la técnica de destilación por arrastre de vapor, y se determinó sus propiedades físicas, químicas y enantioméricas. La caracterización química cualitativa y cuantitativa del aceite esencial se llevó a cabo mediante las técnicas cromatográficas CG-EM y CG-FID en donde se obtuvieron 39 compuestos que constituyen el 97.55% de la muestra total de aceite esencial. Los compuestos mayoritarios fueron: β -Pinene (30.45%), Sabinene (27.98%), α -Pinene (4.97%), Phellandrene $\langle\beta\rangle$ (4.79%), Caryophyllene $\langle E\rangle$ (4.44%), Limonene (3.84%). El análisis enantiomérico del aceite esencial de *L. betonicifolia* mediante CG-EM quiral permitió la identificación de cuatro parejas de enantiómeros: *S* (+) Thujene $\langle\alpha\rangle$ (61.72%), *R* (-) Thujene $\langle\alpha\rangle$ (38.28%); *S* (+) β - Pinene (3.37%), *R* (-) β - Pinene (96.63%); *S* (+) Sabinene (93.56%), *R* (-) Sabinene (6.44%); y *S* (+) Muurolene $\langle y\rangle$ (37.37%), *R* (-) Muurolene $\langle y\rangle$ (62.63%). Se evaluó la actividad antimicrobiana sin presentar actividad considerable.

Método

La especie se recolectó en el barrio Gañil, Cantón Saraguro, provincia de Loja. La extracción del aceite esencial de *Lepechinia betonicifolia* se realizó mediante el método de destilación por arrastre de vapor, durante un tiempo de 4 horas para lo cual se hizo el uso de un destilador tipo clewenger. Se determinó el rendimiento del aceite y las propiedades físicas fueron determinadas según las normas AFNOR NF T75-111 y AFNOR NF 75-11225, para densidad relativa e índice de refracción, respectivamente. Se midió la capacidad del aceite para desviar la luz para lo cual se utilizó el polarímetro automático modelo Hanon P810. Por otro lado, la composición química del aceite esencial de la especie *Lepechinia betonicifolia* se determinó mediante la técnica de cromatografía de gases acoplada a espectrometría de masas, mediante el cromatógrafo de gases de la serie Agilent 6890N acoplado a un espectrómetro de masas Agilent serie 5973 inert, las corridas cromatográficas se hicieron en una columna no polar DB5-MS.

Resultados / Discusión / Conclusión

En la composición química del aceite esencial se identificaron 39 compuestos correspondientes al el 97.55% de la muestra total de aceite esencial. Se identificaron 6 compuestos mayoritarios principales: β -Pinene (30.45%), Sabinene (27.98%), α -Pinene (4.97%), Phellandrene $\langle\beta\rangle$ (4.79%), Caryophyllene $\langle E\rangle$ (4.44%), Limonene (3.84%).

Mediante el análisis enantiomérico se logró determinar 4 pares de enantiómeros: *S* (+) Thujene $\langle\alpha\rangle$ (61.72%), *R* (-) Thujene $\langle\alpha\rangle$ (38.28%); *S* (+) β - Pinene (3.37%), *R* (-) β - Pinene (96.63%); *S* (+) Sabinene (93.56%), *R* (-) Sabinene (6.44%); y *S* (+) Muurolene $\langle y\rangle$ (37.37%), *R* (-) Muurolene $\langle y\rangle$ (62.63%).

Es la primera vez que se reporta la composición enantiomérica del aceite esencial de la especie *Lepechinia betonicifolia*.

El aceite esencial de *Lepechinia betonicifolia* presento un rendimiento de 0,16%, densidad relativa de 0,87 g/cm³, un índice de refracción de 1,48 y rotación óptica de 17,15.

El aceite esencial no presento actividad considerable frente a cepas bacterianas ni en altas concentraciones.

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Composición Química de la Fracción Volátil de la Especie *Valeriana microphylla* Kunth del Sur del Ecuador

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Introducción

La especie *Valeriana microphylla* Kunth pertenece a la familia Valerianaceae, se encuentra en los páramos de pajonal andinos del Sur de Ecuador en un rango altitudinal de 2961 m a 3620 m (Pulgar & Aguirre, 2007). Esta especie en Ecuador es empleada por comunidades indígenas nativas como antiespasmódico, sedante y para reducir el estrés. A partir del extracto en éter de petróleo de las partes aéreas y subterráneas, se han aislado, los lignanos: hidroxipinorresinol y pinorresinol y los valepotriatos: valtrato, iso-valtrato, diavaltrato, acevaltrato y didrovaltrato (Bach, Ghia, & Torssell, 1993). Sin embargo, hasta la fecha no existen investigaciones sobre la fracción volátil de dicha especie. Este estudio es el primer reporte de la composición química del aceite esencial de la especie *V. microphylla*, lo cual constituye un aporte científico para conocer la composición de toda la planta.

La presente investigación es parte de un proyecto de colaboración entre el Consejo de sanadores de Saraguro Hampiyachakkuna, conjuntamente con la Universidad Técnica Particular de Loja y el Ministerio de Salud del Ecuador, cuyo objetivo es estudiar los recursos terapéuticos naturales usados por la comunidad de Saraguro y determinar que recursos pueden ser aprovechados en emprendimientos agroindustriales sustentables.

Método

Se recolectó la parte aérea de la especie *Valeriana microphylla* en la provincia de Loja, Sector las antenas, sur del Ecuador. La extracción del aceite esencial (AE) se la realizó mediante la técnica de destilación por arrastre de vapor en un equipo destilador tipo Cleverger. La destilación duró 3 horas. El AE recolectado fue almacenado en un vial a -4°C. Las técnicas cromatográficas CG-EM (Cromatografía de Gases acoplado a Espectrometría de masas) y CG-FID (Cromatografía de Gases acoplado a Detector de Ionización de la Flama) permitieron realizar el análisis químico cualitativo y cuantitativo del AE, respectivamente. Se utilizó una columna cromatográfica no polar DB5-MS y una polar HP-Innowax. La identificación de los compuestos volátiles fue en base al índice de retención lineal (LRI) y la comparación de los espectros de

masa de cada compuesto con la bibliografía existente (Adams, 2017). En ambos casos se utilizó una serie homóloga de alcanos es de C₉ a C₂₄.

Resultados/Discusión/Conclusión

El análisis químico permitió cualificar y cuantificar la composición química presente en la fracción volátil de la especie *Valeriana microphylla* Kunth, los compuestos mayoritarios identificados son Germacrene D (16.99%), (E)-Caryophyllene (9.08%), α -Copaene (8.36%) y α -Gurjunene (8.00%).

En la columna cromatográfica DB5-MS se logró identificar el 94.13%, de los cuales 2.69% corresponde a monoterpenos oxigenados, 74.41% a sesquiterpenos hidrocarbonados, 1.43% a sesquiterpenos oxigenados y 15.60% a otros compuestos. Un resultado equivalente se encontró en la columna cromatográfica HP-Innowax. No se detectó presencia de monoterpenos hidrocarbonados.

Con los resultados se puede determinar que el AE de *V. microphylla* es de carácter sesquiterpénico y contiene compuestos mayoritarios de gran importancia biológica como lo son Germacrene D, (E)-Caryophyllene, α -Copaene y α -Gurjunene. Actualmente, para complementar el estudio químico se realiza el análisis enantiomérico del aceite esencial, así como ensayos biológicos para determinar la actividad del AE.

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Characterization and classification of cytochrome P450 enzymes present in the *Ganoderma australe* genome, and its association with certain metabolic pathways

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Introduction

New sequencing methodologies have spurred a significant increase in sequenced genomes and biological data, which have permitted the identification of new genes, functional annotation, new metabolic routes, and evolutive associations between species. One gene super family with genes of biotechnological interest is that of cytochrome P450. To date, over 6,000 putative genes have been identified from fungi codified as cytochrome P450 enzymes, and grouped into 276 families. The CYP450 identified in the fungi are the first members of a new family, which suggests that the fungi kingdom represents a source of yet unexplored enzyme families with new catalytic functions. Objective: to identify and classify cytochrome P450 enzymes present in the *Ganoderma australe* genome and determine the metabolic routes associated with this gene super family.

Method

A total of 248 *G. australe* proteins associated with cytochrome P450 were identified in the Pfam database, by way of the Hmmer tool, through alignments with an identity percentage of greater than 40%, and an alignment coverage of 80%, as selection criteria. Exactly 125 sequences were found to have high levels of homology with the different types of fungi sequences from the cytochrome P450 enzyme reference database. Redundancy between sequences was identified, for which reason non-redundant sequences were filtered to 90%, by way of the Cd-Hit (Cluster database at High identity with tolerance), in order to avoid superposition of hits between families.

Results and Discussion

Precisely 44 orthological groups were identified among the *G. australe* and other basidiomycota species sequences, associated with 20 families. Functional annotation was performed via the InterPro program, and in silico validation of the CYP63 family was carried out. Family subdivisions were found on the sequence level, which resulted in the identification of subgroups, which might be associated with other functions within the family. Finally, 25 metabolic routes were identified by way of the KEEG (KASS) automatic annotation tool, and were correlated with enzymes reported in the human species.

Conclusion

The characterization of the cytochrome P450s super family, present in the *G. austral* genome, was initiated, in order to contribute to biotechnological applications which aid in the generation of pharmacological products of natural origin.

Key words: Ganoderma australe, metabolic routes, cytochrome P450, gene super families.

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Observations on traditional usage of ethnomedicinal plants in the tribal belt of the cold desert area of Lahaul valley of Himachal Pradesh, India

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Abstract

Ethnobotanical relevance: The tribal communities of Lahaul valley of Himachal Pradesh have a great faith in *Amchi* system of medicine practiced in the entire region from ages. The *Amchi* system of medicine has similarities with Ayurvedic medicine and includes plants as the key source of drug or treatment strategy. Therefore, extensive field work was conducted to explore information on usage of traditional phytotherapy by tribal communities and healers of Lahaul valley.

Materials and methods: Direct interviews of 150 informants were conducted. The data generated through interviews was analysed using quantitative tools such as use-value (UV), factor informant consensus (F_{ic}) and fidelity level (FI).

Results: A total of 64 plant species in 59 genera and 27 families were observed to be medicinal and used to cure 35 ailment categories. The highest number of ethnomedicinal plants was recorded from the family Compositae followed by Lamiaceae, Apiaceae, Rosaceae, Polygonaceae, Ranunculaceae, Asparagaceae and Crassulaceae. Whole plants and roots or rhizomes were the most frequently used plant part used to treat various ailments followed by leaves and aerial parts. *Arnebia euchroma*, *Artemisia maritima*, *Taraxacum officinale*, *Picrorhiza kurrooa*, *Asparagus filicinus*, *Thymus linearis*, *Podophyllum hexandrum*, *Angelica glauca*, *Rheum webbianum*, *Berginia stracheyi* and *Origanum vulgare* were the most important medicinal plants used for treating various ailments as per use value. The important ailment categories classified on the basis of factor informant consensus were respiratory and gastrointestinal disorders.

Conclusion: This research emphasizes on the documentation of ethnomedicinal plants and their use in *Amchi* system of medicine by tribal communities and healers of Lahaul valley. However, scientific validation is required to determine their efficacy and safety for curing various ailments.

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Inhibition of the oxidation of LDL and human serum with fruits of *Solanum lycopersicum* L. grown with magnetically treated water

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Introduction

Lipid peroxidation is the oxidative degradation of lipids by free radicals. *Solanum lycopersicum* L. contain antioxidant compounds that can neutralize free radicals. They inhibition of oxidation in low density human plasma lipoproteins (LDL) and in human serum was evaluated with hydroalcoholic extracts of *Solanum lycopersicum* L. fruits (tomato), irrigated with static magnetic field (SMF).

Method

The experiments were carried out with the approval of the Ethics Committee of the Federal University of Santa Catarina, in Brazil. A completely randomized experimental design was used with two experimental groups: plants irrigated with tap water (control group) and the other group (WSMF), plants irrigated with water treated with SMF between 20 and 200 mT by means of a treatment device.

With the mature fruits of both experimental groups, hydroalcoholic extracts were prepared at 1, 5, 10, 30, 50, 75, 125, 250, 500 $\mu\text{g mL}^{-1}$. *In vitro* peroxidation was determined in LDL and in human serum induced by copper sulfate. The oxidation of LDL was evaluated by the production of TBARS. The results were expressed as a percentage of copper oxidation protection. In the statistical processing, the normality of the distributions was determined by the Kolmogorov-Smirnov test. To compare the results between the experimental groups, an Type I ANOVA, ($p < 0.05$). The test of comparison of multiple ranges of maximum significant differences of Tukey (HSD) was applied.

Results / Discussion / Conclusion

The hydroalcoholic extracts of the WSMF experimental group, for all the concentrations evaluated, had the highest protection values of peroxidation of LDL and human serum, with respect to the control group, with statistically significant differences ($p < 0.05$). In the WSMF group at 50 $\mu\text{g mL}^{-1}$, the highest percentage of protection to LDL and human serum was achieved, when compared with the rest of the concentrations of this

group. Hydroalcoholic extracts of fruits of *Solanum lycopersicum* L. with water treated with SMF decreased lipid peroxidation in the *in vitro* models used. The irrigation water treated with CME was better assimilated, due to possible modifications in its physical and chemical structural properties, which affected the metabolism of antioxidant active compounds. Probably, also, by the mechanisms of signal transduction in the cell at different levels, influenced by the primary interaction of the magnetic field with the cell membrane. It could be favored the antioxidant, preventive and kidnapping activity. It showed a high protection to lipids against oxidation, and in turn to oxidative stress, a process associated with cardiovascular diseases, neurodegenerative diseases and cancer.

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Peruvian herbal medicines in the context of Nagoya Protocol; challenges and opportunities

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Introduction

It is estimated that the Peruvian Amazon holds more than 1000 plant species with commercial potential and the national sales of natural products derived from medicinal and aromatic plants have exceeded \$ 400 million per year. BioTrade companies such as the Takiwasi Laboratory have focused mainly on the collection, processing and marketing of products based on local biodiversity under sustainability criteria (UNCTAD, 2016). Research and development activities and collaborations are also carried out on the genetic and biochemical composition of the plants, which implies to abide by the national and international regulations, such as the Nagoya Protocol (NP). Despite this richness in terms of indigenous traditional knowledge and biodiversity, Peru, like many other countries, is struggling to update its administrative procedures and regulations in order to respond in a timely manner to researchers and private companies that request formal access to genetic resources.

Method

A global review on the application of the NP with a focus on Peru's situation was performed also through the use of tools such as the Access and Benefit-Sharing Clearing-House (ABSCH). A first-hand experience on the application of the protocol in Peru was also undertaken. Major publications including peer reviewed articles were recorded and discussed.

Results / Discussion / Conclusion

As of May 2019, there have been 440 Internationally Recognized Certificates of Compliance (IRCCs) granted in 16 countries, of the 116 that are party to the NP, that has entered into force in 2014. The main countries of origin are India (220), Spain (40), France (38), Kenya (38), South Africa (27), and Panama (19). That only leaves 58 for the rest of the countries. Peru has issued 3 IRCCs through the 2 identified National Authorities. All the certificates are for non-commercial purposes, although one of these has been signed with



a private company. In this last case the whole process, from initial application to the final access contract signed, lasted around 24 months.

Some of the difficulties observed have to do with the degree of effective implementation of the Access and Benefit-Sharing (ABS) system, the lack of clarity of the application process, and the wide gap between this formal system and what occurs informally outside of it. The most demand for access is concentrated on research, though one of the consequences of the NP seems that researchers from provider countries such as Peru experience increasing difficulties to connect with researchers in developed countries (Deplazes-Zemp et al., 2018). The ineffective application of the NP in Peru could also lead companies and researchers to move their interests to neighbouring countries that have a similar biodiversity and a less demanding regulatory framework, as is the case of Brazil. A further hurdle can often be the reluctance to embrace intellectual property rights, which hinders the essential open dialogue and cooperation between commercial partners and biodiversity-rich countries such as Peru (Heinrich & Hesketh, 2019).

These difficulties inhibit the dissemination of knowledge and genetic resources worldwide. In response to this, training and implementation projects are being launched thanks to international funds so that the Peruvian national framework could be further developed and substantiated according to the (good) spirit of the Nagoya Protocol.

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Activation of the Nrf2 signaling pathway by specioside, an iridoid isolated from the inner bark of *Tabebuia Rosea* (Bertol D.C)

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Introduction

Plants are an important source of biologically active natural products, many of which also constitute models for the synthesis of various drugs. The genus *Tabebuia* includes about 100 species and is the largest genus in the Bignoniaceae family. Several molecules isolated from the inner bark of some species of this genus have pharmacological potential, such as lapachol, α -lapachona and furanonaphthoquinones, among others. A large number of chemical compounds exert their antioxidant effects through the activation of important transcriptional regulatory mechanisms, such as the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2). In cells exposed to oxidative stress, Nrf2 is released and translocated to the nucleus, where it activates its antioxidant response. This study aimed to evaluate *in vitro* the antioxidant activity of Specioside, a compound present in the *n*-butanol extract obtained from the inner bark of *Tabebuia rosea*. This activity is mediated by the nuclear factor erythroid-related 2 (Nrf2), using HepG2 cell line.

Method

Specioside is an iridoid isolated and characterized from the *n*-butanol extract obtained from the inner bark of *T. rosea* by Grupo Polifenoles at Universidad Tecnológica de Pereira. The antioxidant activity of specioside was evaluated using the ORAC and DPPH techniques. The effect of specioside on the viability of HepG2 cells was determined using the MTT method. The effect of the compound on the translocation of Nrf2 to the nucleus was evaluated using the Nrf2 Transcription Factor Assay Kit (Abcam), according to the manufacturer's instructions. Conventional and solvent-guided docking was performed using the AutoDock software.



Results / Discussion / Conclusion

The antioxidant activity shows a significant difference ($p < 0.05$) when comparing the antioxidant activity of the isolated compound with the *n*-butanol extract, and controls (α -lipoic acid and catalposide). The viability was determined using the MTT assay. The results show that specioside did exhibit activity at low concentrations (0.5–2 μM) without affecting cell viability (greater than 90% after 24 hours of exposure), allowing us to perform *in vitro* tests to evaluate the effect of the compounds on the activation of the Nrf2 signaling pathway in HepG2 cells. Nrf2 is dissociated from Keap1 by the *n*-butanol extract at a concentration of 0.5 μM after 4 hours of exposure, showing an increase in cytosolic and nuclear Nrf2 levels with a significant difference ($p < 0.05$) in comparison to the basal levels. At a concentration of 2 μM , an increase in the Nrf2 levels in the cytoplasm after 4 hours of exposure was observed and the Nrf2 levels did stabilize in the nucleus at 12 hours after stimulation with both the specioside and extract, evidencing a significant difference when compared to the basal Nrf2 levels ($p < 0.05$). Molecular docking analysis showed the interaction of the iridoid group of the ligand with the Tyr572 and Ser602 residues of Keap1, necessary for the interaction with Nrf2.

Acknowledgment To Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0572-2012-Bio-Red-Co-CENIVAM, Vicerrectoría de Investigaciones Innovación y Extensión Universidad Tecnológica de Pereira, Fundación Universitaria Autónoma de las Américas and Sistema General de Regalías de Colombia (Código BPIN 2012000100050). for financial support.

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Mazri (*Nannorrhops ritchiana* (Griff) Aitch. a remarkable source of manufacturing traditional handicrafts, goods and utensils in Pakistan

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Abstract

Nannorrhops ritchiana a species of family Arecaceae, native to Pakistan, Iran, Afghanistan, Oman and Saudi Arabia. In Pakistan it is used for various purposes. The species indicates a significant cultural value in the lives of indigenous communities. However uses of *Nannorrhops* are generally known by local populations. In current study we report different uses of *Nannorrhops* and its importance to Pakistani people. Eighty six structured and semi structured interviews including questionnaire data were collected randomly. Seventeen local knowledge holders (12 were male and 5 were female), 23 handicraft experts (21 were male and 2 were female), 19 Shop keepers (all male) and 27 Mazri farmers (all male) were interviewed during field work. The informants' age ranges from 17-83 years old. Uses of different items formed from Mazri palm documented during survey in markets and villages were arranged into use categories and sub categories. Different parts of the plant are currently used by local population e.g, leaf, petiole, fruit and bark. Leaves of the plant are highly used part by the local inhabitants to form different items. Leaves are used in processing of mats, baskets, hand fans, hats, cages, hot pots, salt pots, brooms etc. Mats, baskets and ropes are highly used categories. Mat for grains and prayer mat for group of individual are items with high prices. *Nannorrhops ritchiana* is of immense importance to the local communities of Pakistan. Mazri palm proved to be an important resource for the livelihood of the local communities in providing a lot of handicrafts, food and considerable source of fuel energy. Most informants lacked knowledge about sustainable practices and management of this resource.

Key words: Mazri palm, baskets, sub categories, petioles, handicraft

In vitro inhibition of angiotensin-converting enzyme with extracts of *Jatropha gossypifolia* and *Azadirachta indica*

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Introduction: Hypertension is a chronic multifactorial disease considered as the first cause of death in the world, which appropriately causes 7 million deaths annually, the pharmacological treatment of this pathology, is based on the inhibition of the Renin-Angiotensin System, being one of the main white therapeutic pharmacological inhibition of Angiotensin converting enzyme, this is responsible for converting Angiotensin I into Angiotensin II, a potent vasoconstrictor, that is why he proposed to evaluate the inhibition of the Angiotensin Converting Enzyme with extracts of *Jatropha gossypifolia* and *Azadirachta indica*, as possible therapeutic targets for the treatment of this pathology.

method: Dry plant material of *Jatropha gossypifolia* and *Azadirachta indica* was used to initially prepare methanolic extracts. The inhibition of the Angiotensin Converting Enzyme was evaluated with the method of Cushman and Cheung (1971), which is based on the release of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL catalyzed by the ECA. the ECA kit of Sigma Chemical (St. Louis, MO, USA) whose positive control is captopril, the tests were carried out in triplicate and 2 "blanks" A and C were prepared, in blank A no sample was added (inhibitor) but 0.1M Na-Borate buffer (0.3M NaCl), blank C does not add or sample (inhibitor) or ECA solution, instead, 0.1M Na-Borate buffer (0.3M) is added NaCl) The absorbance was read 228 nm at a temperature of 37 ° C. The results are shown as the mean ± standard deviation (SE), were processed using the GraphPad Prism (version 5.00 for Windows). The analyzed extracts were evaluated by means of the ANOVA test and for all the analyzes the Significance was established at p <0.05

Resultados / Discusión / Conclusión: concentrations chosen to perform the tests were: 1, 10, 100 and 1000 ppm, the highest inhibition of the ACE was obtained by the concentration of 1000 ppm of *Azadirachta indica*, which inhibits 66%, compared to the positive control that inhibited 65%, followed by the concentration of 100 ppm that produced an enzymatic inhibition of 62%, *Jatropha gossypifolia* on the other hand produced a 61% inhibition and 43%, with the concentrations of 100 and 100 ppm respectively, both results below of positive control.

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PERFIL LIPÍDICO DE *Cucurbita foetidissima*

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Introducción

Cucurbita foetidissima (Cucurbitaceae), es una especie silvestre de ambientes xerófitos (Lira, R. *et al* 1998). Representa una planta de interés dado sus diversos usos tradicionales, aunque actualmente existen pocos estudios que permitan identificar su potencial fitoquímico. El presente estudio tiene como objetivo establecer el perfil lipídico en los diferentes tejidos del fruto de *C. foetidissima*.

Método

Frutos maduros de plantas de *Cucurbita foetidissima* se colectaron en la localidad de Ojuelos - Jalisco; se separó tejido de pulpa, semilla y cáscara; a los cuales se pesó 5 g de muestra a cada uno y se les determinó el contenido total de lípidos a partir de extracciones en Soxhlet usando éter de petróleo (hexanos). Con los extractos de hexanos se determinó el contenido de lípidos neutros mediante aplicaciones sobre HPTLC en placas de vidrio (Gel de Sílice 60 F₂₅₄, de 20x 10 cm Merck, Darmstadt, Germany) utilizando un muestreador de TLC automático CAMAG (ATS4) y empleando una fase móvil de éter de petróleo: éter etílico: ácido acético 80:20:1 (v/v/v) a 4°C durante 20 min. El revelado se llevó en una cámara saturanda con yodo; la documentación se realizó con un visualizador CAMAG TLC bajo luz UV a 254nm y 366nm antes y después de la derivatización; los lípidos separados fueron identificados por la comparación de los valores R_f con compuestos de estándares puros. Adicionalmente se determinó el perfil de triglicéridos basado en el método de la Pharmacopeia Europea 2008; así como la presencia cucurbitacinas a partir de extractos etanólicos y basado en Plant drug Analysis - Wagner H. & Blatt S. 2001.

Resultados / Discusión / Conclusión

El mayor contenido de extracto lipídico de *Cucurbita foetidissima*, se obtuvo en las semillas con un volumen de 1.9mL; en la separación de lípidos neutros, este tejido presentó un predominio de triglicéridos, los cuales

fueron comparados con extractos de calabazas domésticas simultáneamente; los que revelaron una similitud en las bandas inferiores con un Rf de 0.19 entre especies domésticas y silvestres. Sin embargo, las bandas superiores a un Rf de 0.24, 0.28 y 0.34 estuvieron presentes principalmente en las especies silvestres lo que puede considerarse como una huella dactilar que muestra las diferencias de las calabazas domésticas. En cuanto a las cucurbitacinas, representan compuestos triperthenos tetracíclicos altamente insaturados que confieren el sabor amargo a muchas especies de la familia Cucurbitaceae; pero son difíciles de detectar por ser solubles en agua y disolverse en lípidos, para el presente estudio, éstos compuestos predominaron en la cáscara y pulpa de la especie evaluada, con Rf inferiores a 0.29 constituyendo cucurbitacinas glucosidadas (Ríos, J. L., *et al.*, 2005).

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Determination of tocopherols in grape stalk using supercritical fluid extraction and ultra high performance liquid chromatography with fluorescence detection (UPLC-FLD)

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Vitamin E is an important natural antioxidant in foods, especially those rich in polyunsaturated fatty acids [1]. Due to its role as a scavenger on free radicals, vitamin E is also believed to protect our bodies against degenerative malfunctions, mainly cancer and cardiovascular diseases [2]. Natural vitamin E is composed of eight chemical analytes: α -, β -, γ and δ -tocopherols and four corresponding tocotrienols. Their chemical structures are shown in figure 1.

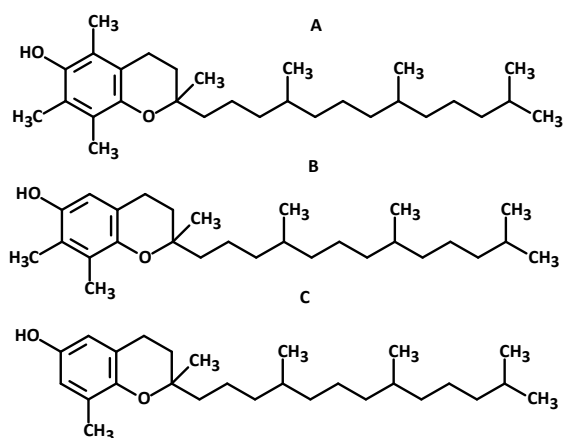


Figure 1. –Chemical structures of α -tocopherol (A); γ -tocopherol (B) and δ -tocopherol (C).

This report describes the development of a method for the extraction of tocopherols in grape stalk using supercritical fluid extraction (SFE), followed UPLC and fluorimetric analysis. The analysis of the selected tocopherols was carried out on an Acquity H-class UPLC system equipped with a quaternary solvent manager, a sample manager, a column heater, a fluorimetric detector and degassing system. Data Handling was managed by Empore v. 3.0 software. Chromatographic separations were carried out using a Fortis SpeedCore C₁₈ (150 x 4.6 mm I.D., 2.6 μ m particle size). Detection was carried out by measuring the



fluorescence at $\lambda_{em} = 328$ after excitation of the analytes at $\lambda_{exc}=303$ nm. The mobile phase was a mixture of methanol and water.

The procedure described is very simple and rapid and should be of value for the quantitation of these analytes in grape stalk.

Evaluation of the trypanocidal effect of extracts obtained from plants of the *Ageratina* genus on *Trypanosoma cruzi*

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Introduction

Chagas disease (ChD) is an unattended tropical pathology caused by the *Trypanosoma cruzi* protozoan. It is estimated that the disease affects about 8 million people in the world (1,2). The drugs (Benznidazole or Nifurtimox) available for the treatment of the disease are controversial because they present several problems associated with the toxicity of the drug, the side effects, the time of treatment, the presence of isolates with different degrees of susceptibility to drugs, low adherence of patients to treatment and poor availability of medications in countries where the disease is endemic (3). In this way, natural products can be considered a potential source of new, effective and selective agents for the development of new drugs in the treatment of ChD (4).

Methodology

The aerial parts of the selected plant species *Ageratina vacciniifolia* and *Ageratina gynoxoides* were subjected to exhaustive successive extraction by maceration using hexanes, dichloromethane and ethanol-water (7: 3) as solvents. The selectivity of the treatments was evaluated in vitro by the MTT colorimetric technique in two stages of *T. cruzi* and in VERO cells. The chemical composition of the extract with the highest activity was evaluated by comparing the fragmentation patterns obtained in the mass-coupled gas chromatography with the available databases.

Results and Discussion

The hexane and dichloromethane extracts obtained from the two plant species showed inhibitory activity of *T. cruzi* epimastigotes stage with IC₅₀ between 66 and 571.3 µg / mL, with the hexanic extract of *A. vacciniifolia* which showed the highest activity against epimastigote stages and trypomastigotes with an IC₅₀ of 66 and 54.33 µg / mL, respectively. The analysis of the major components of this extract showed great diversity of compounds of the terpenoid type, especially sesquiterpenes, of which germacrene D was the

most representative with 11% abundance. However, to date the direct contribution of germacrene D to trypanocidal activity has not been substantiated.

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High Resolution Melting (HRM) genotyping in the genus *Origanum*: Molecular identification and discrimination of the original plant material for authentication purposes

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Introduction

Origanum (family Lamiaceae) is an important genus of medicinal and aromatic plants used since ancient times as culinary herbs and as remedies in traditional medicine. Within the genus, there are numerous species, subspecies, varieties, and hybrids with remarkable morphological and chemical diversity. High Resolution Melting (HRM) analysis, coupled with microsatellite markers (EST-SSRs), could facilitate the molecular identification and characterization of certain genotypes more effectively and relatively faster when compared to other analytical methods.

Method

In this study, twenty-four *Origanum* genotypes corresponding to five *Origanum* taxa (*O. vulgare* subsp. *hirtum*, *O. lirium*, *O. majorana*, *O. onites*, and *O. dictamnus*) were analyzed, using six microsatellite loci. Our goal was to molecularly identify and to discriminate among the selected genotypes and to evaluate the ability of the HRM technique as an analytical tool.

Results/Discussion/Conclusion

The temperature-shifted melting curves produced by the HRM analysis, resulted in 54 unique HRM profiles, which enabled the discrimination of the *Origanum* genotypes studied. According to the similarity dendrogram based on the HRM profiles, two major clusters were formed. The first cluster consisted of *O. onites* together with *O. dictamnus* genotypes, while the second was divided into two sub-clusters, one comprising the two *O. majorana* genotypes, while the other those of *O. lirium* and *O. vulgare* subsp. *hirtum*. In conclusion, HRM genotyping provided a fast, cost-effective method, well suited for the molecular characterization and identification of *Origanum* taxa and for the authentication of the original genetic material.

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Synthesis of new steroidal heterocyclic compounds

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Introduction

Steroids are an important group of natural compounds exhibiting important and diverse biological, chemical and pharmaceutical applications which are extensively available in animals and plants.¹

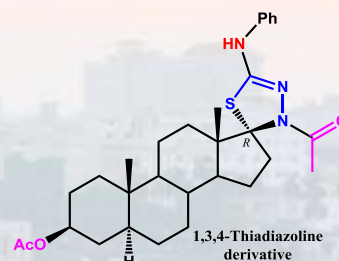
Numerous steroidal heterocycles have been synthesized and studied in recent decades, due to the important biological activities that they possess, for example antitumor, anti-inflammatory, anabolic, androgenic,² antibacterial, antiproliferative, cytotoxic,³ or anticancer activity.⁴ Five member heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics, etc. Compounds with a thiadiazoline structure are known to possess tranquilizing, muscle relaxing, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial, antifungal, analgesic, and antiinflammatory properties.^{5,6} The fusion with heterocycles that have one or more heteroatoms in the A-D rings of the steroidal skeleton confer diverse interesting pharmacological activities. Some of them evaluated as inhibitor of growth in human carcinoma cell lines.⁷⁻⁹

Method

The intermediate thiosemicarbazones were prepared by reacting the carbonyl functionality C-17 of estrone, *trans*-androsterone and dehydroandrosterone with 4-phenylthiosemicarbazide. After that, the novel spiroheterocycles on the D ring of steroidal skeleton were synthesized by intramolecular oxidative cyclization according to the literature procedure via acetylation of steroidal thiosemicarbazone derivatives. All the synthesized compounds were characterized by physical, spectroscopic methods and X-ray diffraction in some cases. From the literature no work has been done on estranes and androstanes derivatives screening as antiproliferative agents.

Results / Discussion / Conclusion

One-dimensional and two-dimensional spectral NMR analysis were utilized to make the complete assignments for all compounds. It was possible to determine that intramolecular cyclization in basic medium does not take place stereoselectively when starting material is estrone. The reaction generated a mixture of



epimers, ^{13}C NMR spectrum evidenced the formation of spiro-carbon atom, which revealed two signals at ~90 ppm. It could be established that the methyl group C-18 does not influence the nucleophilic addition step of the sulfur atom on the C=N bond.

The thiosemicarbazone derivative from *trans*-androsterone on reaction with Ac_2O and Py at 60 °C gave a major isomer. Suitable crystals were analysed for X-ray crystallography. Whereby, isomer formed has a *R* configuration where the attack of the bulky sulphur atom proceeds on the sterically less hindered face, minimizing the steric interactions of the formed spirocycle. This research involves the synthesis of novel D-ring fused steroidal thiadiazole by cyclization of steroidal thiosemicarbazones from estrone and androstanes. Further biological activity of the compound is in progress.

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Assessment of the genotoxic potential of a methanolic extract from roselle (*Hibiscus sabdariffa* L.) *in vivo*

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Introduction

The red calyces of *Hibiscus sabdariffa* L., commonly known as roselle or jamaica, have received considerable attention in the nutritional field (especially in the preparation of fresh water, desserts and gelatins) and for their various medicinal uses (mainly, antidiabetic and antihypertensive). However, there are contradictory data on the possible alterations that may be exerted on the DNA. The aim of the present study was to determine the clastogenic potential of a methanol extract of roselle (MER) by the micronucleus (MN) assay in male CD-1 mice.

Method

Initially, the lethal dose 50 of MER was determined by the Lorke technique (1983). The test was carried out in two steps: The first doses tested were 10, 100 and 1000 mg/kg and they showed no lethality among the animals. In the second dose interval (1600, 2900, 4300 and 5000 mg/kg) the same effect was observed, no mortality was seen either. With this result, it was considered to use doses of 250, 500 and 1000 mg / kg for the determination of the genotoxic capacity. For which, animals with an average body weight of 20 ± 2 g were used. We included a negative control, a positive batch [cyclophosphamide (CP) 30mg/kg], and three batches of the methanol extract (250, 500 and 1000 mg/kg).

The CP was administered only once intraperitoneally, unlike the extract that was administered orally for 5 days. Later, at different times (0, 24, 48, 72 and 96 h), we quantify the body weight of the animals and performed blood smears, which were stained and observed under the microscope to quantify the number of micronucleated normochromic erythrocytes (ENCMN). Likewise, it was determined the relationship of polychromatic erythrocytes (EPC) with respect to the normochromic erythrocytes (ENC).

Results

At the end of the study, it was confirmed that CP is a significant clastogenic agent whose maximum effect is observed 48 hours after treatment. On the contrary, the extract *per se* does not alter the body weight and did



not increase the frequency of ENCMN in any of the doses and the evaluation schedules. With regard to the cytotoxicity index ($CI=EPC/ENC$), it was observed that MER does not alter the EPC number.

Conclusion

These results suggest that MER is not a clastogenic or cytotoxic agent in the doses evaluated. It is convenient to explore new in vivo studies to evaluate their antigenotoxic capacity against different mutagens and/or carcinogens.

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Chemical composition and enantiomeric analysis of the essential oil from *Piper coruscans* Kunth (Ecuador).

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Introduction

In Ecuador, the genus *Piper* includes 215 species, of which 75 are endemic (Jorgensen & León-Yáñez, 1999). *P. coruscans* is a shrub or treelet of Ecuador (1000-4000 m) (Jorgensen & León-Yáñez, 1999), locally named as “*matico*”. A decoction of leaves mixed with alcohol is drunk for respiratory ailments, digestive disorder and as an anti-inflammatory. (Chamba, 2012). This study describes the GC-FID, GC-MS, and enantioselective GC analysis of the essential oil hydrodistilled from leaves of *P. coruscans* Kunth, collected in Ecuador.

Method

The leaves of *Piper coruscans* was collected in a rain forest habitat in the Zamora Chinchipe Province, Ecuador, in april 2018. The essential oil was obtained by preparative steam distillation and analytical Clevenger-type microscale distillation. The identification and quantification of the oil components was made by CG-EM and CG-FID. The enantioselective analysis was realized by gas chromatography with enantioselective column. Retention indices were determined, according to (Van Den Dool and Kratz, 1963), on the basis of the retention times of a homologous series of hydrocarbons C₁₀-C₂₅ (TPH-6RPM of CHEM SERVICE), which were analyzed by GC under the same conditions.

Results / Discussion / Conclusion

GC-FID and GC-MS allowed the characterization and quantification of 58 components, representing 97,8 % of the total sample. Sesquiterpene hydrocarbons (54,6%) and oxygenated sesquiterpenes (19,1%) were found to be the most abundant volatiles, while the monoterpene hydrocarbons (5,1%) and oxygenated monoterpenes (5,0%) were the minor components. The most abundant compounds were: (*E*)-caryophyllene (24,5%), α -Humulene (11,8 %), caryophyllene oxide (10,1%) and Linalool (4,8%). In the floral water 12 compounds were identified, 77% are oxygenated monoterpenes, 10% oxygenated sesquiterpenes, and 70% other compounds. The two major compounds were: Linalool (78,0%), and α -Terpineol (4,8%). In the enantiomeric analysis 5 pairs of enantiomers were found: (*S*)- α -pinene and (*R*)- α -pinene, (+)- β -pinene and (-

)- β -pinene, (*R*)- α -phellandrene and (*S*)- α -phellandrene, (*R*)-Limonene and (*S*)-Limonene, (*R*)-Linalool and (*S*)-Linalool. No information on the volatile fraction of the *Piper coruscans* was found in the literature, however in many studies conducted in several genera Piper has been found as main constituents sesquiterpenes, monoterpenes and phenylpropanoids (Bottia et al, 2007; Sanchez et al, 2014)

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Nemorosone inhibits macrophage-induced growth of human colon carcinoma cells

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Abstract

Although immune cells are responsible for physiological responses and tissue homeostasis, numerous studies indicate that they could promote tumor progression, invasion and metastasis, establishing a symbiotically relation with cancer cells. Many tumors recruit monocytes from circulation inducing their transition to tumor-associated macrophages (TAMs) through survival factors; in turn, TAMs release angiogenic factors in response to the tumor microenvironment (TME), promoting tumor invasion (1). Several studies have demonstrated the biological properties of Nemorosone (NEM), a polyisoprenylated benzophenone isolated from botanical specie *Clusia Rosea* and present in diverse sample of from Caribbean propolis (2, 3). The antiproliferative activity of this natural product towards different cancer cells have been investigated in detail (4-6), however its effect on TME is unknown.

This study aims to explore the effect of NEM on regulating macrophage polarization and mediating anticancer in tumor microenvironment. Acute monocytic leukemia cell line THP-1 were induced to differentiate into M2-like macrophages (M2). Changes involved in THP-1 differentiation and the effect of NEM (5, 10, 25 and 50 μ M) on THP-1 polarization were evaluated by immunofluorescence analysis and qRT-PCR. In addition, human colorectal adenocarcinoma cell line (HT-29) was cultured in conditioned medium from M2 (M2-CM) with NEM (5, 10, 25 and 50 μ M) to investigate the effect of this natural product on M2-promoted tumor growth, migration and invasion of colorectal adenocarcinoma cells. HT-29 cells proliferation was determined through MTT assay; meanwhile, cell migration and invasion were analyzed by wound healing test and Boyden chamber assay respectively.

Nemorosone significantly inhibited M2 polarization of macrophages, decreasing the expression of specific cell surface markers and M2-associated genes. Moreover, NEM suppressed the M2-induced migration and invasion of HT-29 cells. These preliminary studies suggest that nemorosone blocks the M2-polarization and reduces M2-induced proliferation of HT-29 cells. Therefore, nemorosone may represent a natural product with an intriguing effect on the cancer cells -TAMs relationship.

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Effect of drying process in the bioactive compounds extraction of agroindustrial by-products by supercritical fluids

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Introduction

In order to give an integral use to fruits, it would be beneficial if their waste could be used as a source of bioactive compounds for functional foods. Mango (*Mangifera indica*) and pineapple (*Ananas comosus*) are two of the most consumed fruits in the world. Specifically in peel, numerous phytochemical compounds have been identified with important health benefits. For this reason, the objective of this study was to evaluate both the effect of the peel drying in the extractive process, as well as the optimal conditions of extraction by supercritical fluids (SFE).

Method

Two drying methods were evaluated: lyophilization and vacuum, and for the SFE optimization process the CO₂ flow (1000 mL / h), sample size (3g) and dynamic time (75 min) were fixed while variables pressure (100-300 bar), temperature (30-50 °C) and percentage of co-solvent (ethanol 5-15% of CO₂ flow) were evaluated. Subsequently, the obtained extracts were dried and they were determined yield and antioxidant activity through the methodologies ABTS, DPPH and Total Phenols. Likewise, they were analyzed by HPLC to quantify their content of gallic acid (GA), ellagic acid (EA), quercetin (Q) and mangiferin (M) in the mango case. In order to evaluate the effect of extraction process variables and the dry method on the performance and concentration of bioactive compounds, a composite central design was carried out. Statgraphics Centurion XVI.I software (USA) was used to analyze the results.

Results / Discussion / Conclusion

Regarding the drying process, for mango better results were obtained drying the peels under vacuum, while the optimum of extraction process was found at 300 bar, 30°C and 5% co-solvent. The yield obtained was 21,77% and in terms of antioxidant content the result was: ABTS 59838,74 TEAC (µmol Trolox / 100g DM (DM = dry matter)), DPPH 86379.06 TEAC and Total phenols 4323.53 mg AG / 100g DM. In HPLC, 12.21 µg / mL AG, 534.96 µg / mL M, 23.38 µg / mL AE and 5.52 µg / mL Q were quantified. For pineapple, the drying process that less affected the bioactive compounds was lyophilization. In this case, the SFE optimum was 100 bar, 40°C and 10% co-solvent. The yield obtained was 21,4%, ABTS 9681.11 TEAC, DPPH



9901.24 TEAC and total phenols 1746,51 mg AG / 100g DM; in HPLC 1.68 μg / mL AE, while the other compounds were not detected. The drying process is an important factor in the SFE extraction of the evaluated bioactive compounds. The peels dried by lyophilization present greater porosity and therefore are more hygroscopic compared to those dried in vacuum. The results confirm that these by-products are an important source of bioactive compounds and can be an interesting alternative as a valorization strategy to be used as an ingredient or in the production of functional ingredients.

Keywords: Bioactive compounds, agroindustrial by-products, Honey Gold, Tommy Atkins.

Development of green and automated method for the extraction of nutraceutical compounds from cocoa (*Theobroma cacao*) pod husk

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Cocoa pod husk (CPH) is the main by-product of the chocolate industry. Unfortunately this by-product is discarded, not exploited and scattered among the cocoa plantation until it rots. However, because of its content in bioactive compounds such as theobromine, caffeine and phenolic compounds it could be used for human/animal feeds or industrial applications. In the present work, a pressurized liquid extraction (PLE) was used to develop a rapid, green and low cost process aimed at obtaining an extract enriched in nutraceutical compounds. A Box-Behnken design was used to investigate the effect of three independent variables (temperature, extraction time and co-solvent flow rate) on extraction yield, total phenolic content (TPC) and antioxidant capacity (DPPH assay). Temperature and co-solvent flow rate were the main factors that influenced the extraction yield of the target compounds. The results showed that PLE is an efficient and reliable technique for the valorization of an underexploited, readily available and low cost materials such as CPH through recovering of bioactive compounds that can be used for food, cosmetic or pharmaceutical applications.

***Rosmarinus officinalis* L. cultivated with magnetically treated water and the hepatoprotective activity**

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Introduction

In Cuba, liver diseases represent the ninth cause of death. In Cuba, liver diseases represent the ninth cause of death. Since 1980, the prevalence of non-alcoholic fatty liver disease (NAFLD) has increased rapidly, associated with the dramatic increase in obesity and diabetes in the world. Currently NAFLD is considered as the most common cause of chronic liver disease in the Western world, as well as Latin American countries, closely related to the metabolic syndrome. The aim of this study was to evaluate the protective effect of *Rosmarinus officinalis* L. cultivated with magnetically treatment water on acetaminophen induced liver damage.

Method

For magnetic water treatment, magnetizer with permanent imam was applied in the pipe way to the field. Magnets were designed, built and calibrated at the National Center of Applied Electromagnetism (CNEA), with the characteristics of 10 cm device and magnetic induction between 0.06-120mT.

The aqueous extracts were obtained from plants irrigated with magnetically treated water (MTW) and plants without magnetically treated water (control). There were four experimental groups by mice OF-01, group 1 was administered with distilled water, group 2: it was administered with acetaminophen to 400 mgkg⁻¹ corporal weight, group 3: it was administered with 100 mgkg⁻¹ corporal weight with the aqueous extract from plants without MTW and group 4: it was administered with 100 mgkg⁻¹ corporal weight with the aqueous extract with plants MTW.

All groups were evaluated for mortality percentage, the best groups between aqueous extract of plant irrigated with static magnetic fields, the following was determined the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) enzymes, Malonaldehyde concentration.



Results / Discussion / Conclusion

The results demonstrated that the low mortality was in the aqueous extract of plant SMF in compared with the aqueous extract of plant without SMF, then to the group of plant SMF was obtained a reduction in blood of biochemical parameters like the enzyme level of ALT, AST, ALP, lower malonaldehyde concentration and a higher glutathione and catalase antioxidant enzymes in compared with the group that used acetaminophen induced liver damage. There results suggest that the aqueous extract of *R. officinalis* L. irrigated with SMF can preventive a hepatic damage by acetaminophen. The hepatoprotective activity of the extract in this model seems to be mediated at least partly by its antioxidant properties.

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High-Resolution LC-MS/TOF Chemical Characterization of Bioactive Extract from *Baccharis trimera* (Less.) DC. (Asteraceae)

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Introduction

The genus *Baccharis* contains about 500 species and belongs to the family Asteraceae, one of the most important Angiosperms from the chemical and pharmacological points of view. *B. trimera* (Less.) DC. is a shrub and in Brazil it is known as carqueja. Its medicinal use reports from 1931, and this plant has been used for the treatment of liver dysfunctions and stomach problems (Karan et al., 2013). *B. trimera* was included in the Herbal Compendium of the Brazilian Pharmacopoeia as anti-dyspeptic (ANVISA, 2018). Recent studies performed by the group showed the *in vivo* effect of the aqueous extract from leaves on delayed gastric emptying (Wistar female rats, 50 mg/ kg). The bioactive aqueous extract was submitted to chemical investigation by high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometer by time of flight identification (MS/ TOF).

Method

B. trimera was collected in the the Social and Environmental Responsibility Center at Rio de Janeiro Botanical Garden (GPS S22°58'08" O43°13'03") in March 5th, 2018. Voucher sample was registered under the number RB00641858. This scientific research was recorded in SisGen and was assigned as A3CA014. The fresh plant material was dried under immersion in liquid nitrogen, fragmented and subjected to extraction by infusion in purified water (3% w/ v), as determined by the Herbal Compendium of the Brazilian Pharmacopoeia. The aqueous extract obtained was lyophilized (LAE) and 100 ppm of this extract was prepared for analysis by HPLC in a Shimadzu Prominense coupled to a Bruker micrOTOF II High performance mass spectrometer. The HPLC mobile phase consisted of acidified ultrapure water and acetonitrile, in gradient mode, flow rate at 0.5 mL/ min, oven temperature in 30 °C, injection volume of 20 μ L and UV monitoring at 340 nm. The mass spectra were obtained by ESI in negative mode, with collision energy of 10 and 50 eV, nitrogen volume of 9L/ min, at 200 °C and mass scanning range of 50 to 1,000

u.m.a. Compounds identification were achieved by the respective mass spectra obtained with exact mass and comparison with the database MassEuropeanDataBank, as well as with mass records of compounds already described in the literature for *B. trimera*. Ionization at 50 eV produced fragments from the molecular ion that aided in the confirmation of the compounds.

Results / Discussion / Conclusion

Considering exact masses, fragmentation profile and retention time it was possible the identification of sugars (glucose and sucrose), high percentage of quinic acid (43%), caffeoylquinic and feruloylquinic acids, mono-, di- and triglycosides of apigenin and kaempferol, luteolin monoglycosides and non-glycosylated flavonoids, as well as two polar steroids. Analysis by LC-MS/TOF allowed identification of the chemical composition of aqueous extract from leaves of *B. trimera* (carqueja) that has action in the delay of the gastric emptying.

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Actividad antimicrobiana y compuestos fenólicos en dos especies de *Phyllanthus* cubanas

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Resumen:

Diversas especies del género *Phyllanthus* se utilizan en medicina natural. Múltiples estudios in-vitro e in-vivo comprueban propiedades antiviral, antitumoral, antiinflamatoria y antimicrobiana. Investigaciones demuestran que el origen de la actividad antiviral de estas especies se debe a la presencia de compuestos de naturaleza fenólica que inhiben la ADN-polimerasa y reverso transcriptasa en la réplica de los ADN. Estudios realizados en dos especies endémicas de Cuba demuestran actividad antiviral evidenciadas por: inactivación del antígeno de superficie de la Hepatitis viral B, inhibición de la multiplicación del virus del herpes simple tipo 2 (HSV-2) en fibroblastos de prepucio humano (FPH) y del virus del herpes bovino tipo 1 (BHV-1) en células de riñón bovino (MDBK). La posibilidad de actividad antimicrobiana de extractos con propiedades antivirales le proporcionaría un valor agregado para el uso por vía tópica puesto que muchos microorganismos resultan oportunistas por infectar regiones de la piel lesionada. El trabajo tiene como **objetivo** estudiar la acción antimicrobiana de extractos acuosos de *Phyllanthus chamaecristoides* ssp *baracoensis* Urb. (Webster) y *Phyllanthus orbicularis* HBK. frente a cepas de referencia de organismos patógenos como: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* y *Candida albicans*, aparejado a la cuantificación de compuestos fenólicos presentes en estos. **Materiales y métodos.** Para la actividad antimicrobiana frente a los organismos se utilizó la técnica de microdilución en caldos. La cuantificación de los compuestos fenólicos se efectuó mediante técnica colorimétrica por formación de complejo coloreado por reacción con sal férrica en medio alcalino con lectura de absorbancia a 490 nm. Como **resultados** se obtuvo que para ninguno de los extractos hubo una inhibición significativa que permita correlacionar actividad antimicrobiana con la concentración de compuestos fenólicos. No se descarta la posibilidad de la acción antimicrobiana de estas plantas pues fracciones de baja polaridad resultan positivas para otras especies.

Determinación de compuestos fenólicos totales, capacidad antioxidante y actividad antimicrobiana en corteza de encino (*Quercus* sp.)

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INTRODUCCIÓN

La periodontitis es una inflamación de la encía causada por escasa higiene bucal que, al no ser tratada, el paciente puede perder órganos dentales y presentar reabsorción ósea. En México esta enfermedad tiene una incidencia cercana al 90% lo cual, la convierte en un problema de salud pública así, es importante encontrar una alternativa natural para tratar la enfermedad. Por otro lado, los encinos o robles pertenecen al género *Quercus*, familia Fagaceae; son árboles y arbustos de hoja ancha y dura, su fruto característico es la bellota. Viven en las partes bajas de las montañas mezclados con varias especies de pinos. En México existen más de 200 especies (De Lourdes, et al., 2003), ubicados en el centro y sur del país. Se conocen dos tipos de encino: leñoso, que se utiliza en la producción de madera y encino no leñoso, poco valorado. Sin embargo, para ciertos grupos étnicos tiene aplicaciones medicinales, que son atribuidas a su contenido en compuestos fenólicos, que engloba a todas aquellas sustancias que tienen como base en su estructura al fenol. A este tipo de sustancias se le han asociado propiedades terapéuticas como antimicrobianas, antioxidantes, antiparasitarias, anticancerígenas, astringentes y cicatrizantes. Por todo lo anterior, el objetivo del presente estudio fue evaluar la concentración de compuestos fenólicos en corteza de encino y relacionarlo con su actividad antioxidante y antimicrobiana.

MÉTODOS

Material Vegetal y Preparación de los extractos

La muestra de corteza de encino (*Quercus*) se adquirió de la región de Tlatlauquitepec, Puebla. La muestra recolectada se secó en estufa a 85° C durante 24h y se almacenó en un lugar seco protegido de la luz. La muestra seca se pulverizó y a partir de esta se obtuvieron dos extractos, extracto etanólico de encino, ENC-OH y extracto acuoso de encino, ENC-Ac. Ambas muestras se dejaron reposar por 7 días, en frascos ámbar con cierre hermético en un lugar fresco y protegidos de la humedad.

Determinación de compuestos Fenólicos

Se evaluó la concentración de compuestos fenólicos en el ENC-OH y en el ENC-Ac. Para esto, se siguió el método descrito por (Dastmalchi et al. 2007). Las absorbancias de las muestras se leyeron a una longitud de onda de 725 nm. Para el cálculo de las concentraciones se realizó una curva de calibración con ácido gálico como estándar. Los datos se reportan como mg de Ácido gálico por 100 g de corteza.

Capacidad antioxidante

Para realizar la evaluación de la capacidad antioxidante se utilizó el método de decoloración del reactivo DPPH (2,2- difenil-1-picrilhidracilo) siguiendo la metodología descrita por Brand-Williams et al. (1995). La lectura de las absorbancias se realizó a 515 nm. Los datos se expresan como el porcentaje de inhibición del DPPH (%INH DPPH).

Actividad antimicrobiana

Para evaluar la actividad antimicrobiana de los extractos se probó contra cuatro cepas bacterianas por el método de difusión en agar (Bhunja et al., 1988). Los extractos a probar se colocaron dentro de pequeños pozos realizados en el agar inoculado, las cajas petri se incubaron por 24 h para posteriormente, medir el halo de inhibición para cada microorganismo evaluado. Se utilizó cloranfenicol y solución salina como controles positivo y negativo respectivamente.

RESULTADOS

Tabla 1. Contenido de compuestos fenólicos y capacidad antioxidante de los extractos. Los resultados se muestran como el promedio de tres mediciones \pm el error estándar.

Extractos	Compuestos Fenólicos Totales mg AG/ 100g corteza	Capacidad Antioxidante %InhDPPH
ENC-OH	129.88 \pm 9.64	89.59 \pm 0.43
ENC-Ac	37.68 \pm 85.48	90.88 \pm 0.26

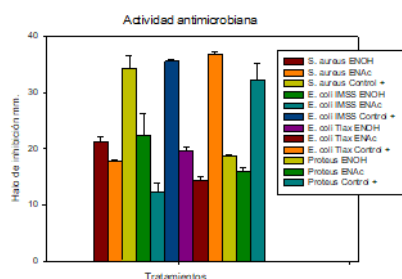


Figura 1. Actividad antimicrobina de los extractos. Los resultados se muestran como el promedio de tres mediciones \pm el error estándar.

DISCUSIÓN

Respecto a los resultados obtenidos, podemos mencionar que el extracto etanólico presentó mayor contenido de compuestos fenólicos que el extracto acuoso sin embargo no sucedió lo mismo al comparar la capacidad antioxidante, ya que en este caso el extracto acuoso en el cual, se aplica temperatura presentó mayor poder antioxidante.

CONCLUSIONES

El extracto acuoso presentó mayor capacidad antioxidante.

El extracto etanólico presentó mayor actividad antimicrobiana contra los microorganismos evaluados.

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Evaluación y comparación de la composición corporal de mujeres adultas mestizas y Wixaritari del Occidente de México

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Introducción

La obesidad se considera un factor de riesgo para el desarrollo de enfermedades crónicas no transmisibles como son; diabetes, síndrome metabólico, hipertensión, cáncer y enfermedades cardiovasculares entre otras (ayers y cols. 2019). En México, el sobrepeso y la obesidad en la población mexicana adulta se encuentra en ascenso según la Encuesta Nacional de Salud y Nutrición de Medio Camino 2016. Siendo la población femenina adulta la que presenta niveles más altos de prevalencia en sobrepeso, obesidad y obesidad abdominal (Instituto Nacional de Salud Pública, 2016). Se han realizado análisis donde se asoció la inseguridad alimentaria como factor de riesgo en mujeres adultas (Morales-Ruán M. C. y cols., 2014), sin embargo no se ha evaluado si las características de la raza/etnicidad puede ser un factor importante para la presencia de obesidad en las mujeres adultas mexicanas.

La región occidente del país es una zona multicultural compuesta principalmente por mestizos y la etnia originaria Wixárika. Las características, climáticas, demográficas son similares para ambas poblaciones (Contreras J. de J., 2016), por lo que nos permite evaluar si existe una diferencia en la composición corporal de mestizos y Wixaritari en igualdad de condiciones. Sin embargo, existe controversia sobre el tipo de métodos y parámetros empleados para evaluar la composición corporal entre diferentes etnias/razas, en particular si el índice de masa corporal puede ser un buen biomarcador predictor del exceso de masa grasa (Heymsfield S. B. y cols. 2016). Por lo que el objetivo del presente trabajo es evaluar y comparar la composición corporal de mujeres adultas wixaritari y mestizas. Además determinar si el índice de masa corporal es eficiente como predictor de exceso de masa grasa para ambas poblaciones.

Método

Se evaluó un total de 68 mujeres adultas de 20 a 85 años de la zona norte de Jalisco, 25 de la etnia original Wixárika y 43 mestizas. En el análisis de la composición corporal se tomó en cuenta las variables; Edad, altura, peso, índice de masa corporal, masa musculo esquelético, masa y porcentaje de grasa corporal, índice-cintura cadera, para su evaluación se utilizó la técnica de bioimpedancia con una báscula portátil de la marca Inbody modelo 120. Se utilizaron los criterios de clasificación de la OMS para evaluar el estado nutricional. El análisis de la normalidad de las variables, se realizó por la prueba de Kolmogorov-Smirnov, las comparaciones por la prueba de t Student y la correlación de las variables, se realizó con la prueba de Pearson todos los datos se presentan como media \pm desviación estándar y se fijó el nivel de significancia con una $p < 0.05$.

Resultados / Discusión / Conclusión

La población mestiza presenta valores superiores a los de la población Wixárika, así como la presencia de sobre peso y obesidad. Sin embargo, solo se encontraron diferencias significativas entre ambas poblaciones para las variables altura (149.5 ± 4.047 vs 156.6 ± 6.011) y masa musculo esquelética (19.1 ± 2.723 vs 23.76 ± 3.781), con una $p < 0.05$. Se observó una correlación significativa ($p < 0.05$), entre el índice de masa corporal y las variables de porcentaje y masa grasa en ambas poblaciones. Los resultados muestran que la composición corporal de la población wixárika es similar a lo reportado, no obstante en la población mestiza se observaron valores por arriba de la media nacional. Por otra parte, el índice de masa corporal si es un biomarcador predictor de la masa grasa en ambas poblaciones, sin embargo falta ampliar el estudio para tener datos confiables. En conclusión, en la zona norte de Jalisco la población mestiza presenta un mayor riesgo de salud, en comparación con la población wixárika. Sin embargo, falta contrastar los datos obtenidos con parámetros bioquímicos, para describir de forma más precisa el estado de salud de las mujeres adultas del Occidente de México.

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Inhibition of cholinesterases by *Petivera alliacea* L.

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Introduction

Among several variants of degenerative dementia, Alzheimer's disease (AD) is by far the most prevalent, affecting more than 20 million people worldwide¹. One of the causes of this disease is low levels of the neurotransmitter acetylcholine (ACh) in the hippocampus and progressively throughout the cerebral cortex, which typically contributes to memory loss and cognitive failure². The liberated ACh has a short half-life in the synaptic groove, due to the presence of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), both enzymes presents in the brain³. Also, both enzymes have been shown to participate in amyloid plaque formation and deposition, and there is evidence that inhibitors of both AChE and BChE, have a beneficial effect on treating mild-to-moderate AD³. *Petiveria alliacea* L. (Phytolaccaceae) is a perennial herb commonly known as “hierba del zorrillo” or “anamú”. In indigenous communities of Brazil, it is used as an enhancer of memory and it is known that it improves short and long-term memory⁴. In this work we studied the effect of organics extracts of *P. alliacea* on activity of AChE and BuChE.

Method

Leaves of *P. alliacea* were collected at Catemaco, Veracruz, Mexico. The dried and crushed leaves were macerated in methanol for three days at room temperature. The solvent was evaporated under vacuum. The extract was subsequently fractionated with solvents of increasing polarity (n-hexane, ethyl acetate and methanol). The inhibitory effect of the extract and fractions on the activity of acetylcholinesterase and butyrylcholinesterase in rat plasma was spectrophotometrically performed with a modification of Ellman's method⁵, using acetylcholine and butyrylcholine as substrates. Methanol extract and its fractions were evaluated at five concentrations (125, 250, 500, 1000, 2000 µg/mL). A reversible acetylcholinesterase inhibitor (eserine at 1 µM) was included as positive control. The reactions were followed at a wavelength of 405 nm.

Results / Discussion / Conclusion

The evaluation of inhibition by n-hexane and ethyl acetate extracts on plasmatic cholinesterase activity, using butyrylcholine as substrate, showed an inhibition of 51% and 83%, respectively, at 500 $\mu\text{g/mL}$, whereas at 1000 $\mu\text{g/mL}$ inhibition was 67% and 82%, respectively. On the other hand, using acetylcholine as substrate, the methanol extract and its methanolic fraction, at 2000 $\mu\text{g/mL}$, inhibited cholinesterase activity by 41% and 42%, respectively. The inhibition observed in this work, may be due to the presence of terpenes in n-hexane and ethyl acetate extracts, which may have preference over the inhibition of butyrylcholinesterase. While, flavonoids, major constituents in the methanolic extracts, seem to have greater inhibitory activity on acetylcholinesterase. Both terpenes and flavonoids compounds with inhibitory effect on cholinesterase have been reported to modify memory, learning and cognition, so metabolites of similar nature in *P. alliacea* could be useful in pathologies such as Alzheimer disease.

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***Lippia origanoides* essential oil induces anti-inflammatory activity *in vitro*. Evaluation of the signaling pathways involved.**

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Introduction

Inflammation is a complex process of events that occurs in response to harmful stimuli or infection and is regulated by the coordinated response of cells and their secreted pro-inflammatory mediators such as nitric oxide (NO), prostaglandin E₂ (PGE₂), tumor necrosis factor α (TNF- α), interleukin (IL)-1 β , and IL-6. Although this response is helpful for the host, persistent inflammation leads to the development of chronic inflammatory-related diseases such as asthma, arthritis, cancer, and neurodegenerative disorders. Non-steroidal anti-inflammatory drugs and COXIBs are currently used to treat acute or chronic inflammatory processes. However, these drugs are associated with gastrointestinal and cardiovascular adverse reactions. Natural products have been used in traditional medicine to control inflammatory-related diseases, and some of their components are responsible for this activity. In search of new anti-inflammatory molecules, this study aimed to characterize the EOs from *Lippia origanoides* and to evaluate the cellular mechanism responsible for this activity^[1] in RAW 264.7 macrophages.

Methods

The components of the EOs were determined by GC-MS, GC-FID, and LC-MS analysis and by comparison of their mass spectra, and linear retention indexes (LRI) with those from standard substances and from different mass spectral bases and data from the scientific literature. The effect of EO of *L. origanoides* on the LPS-induced production of NO, PGE₂, IL-1 β , TNF- α , IL-6 and IL-10 were determined using enzyme-linked immunosorbent assays; the expression of *iNOS*, *COX2*, *IL1B*, *TNFA*, *IL6* and *IL10* genes were measured by qRT-PCR; whereas the effect of EO on LPS-stimulated phosphorylation of MAPK and NF- κ B pathway



proteins such as: Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38, inhibitor protein of NF- κ B (I κ B α) were measured using Western blot. The effect of extracts on cell viability was determined using the MTT assay.

Results / Discussion / Conclusion

The results did indicate that none of the extracts affected cell viability at final concentrations between 0,5 and 2 μ g/mL. The EO did inhibit significantly PGE₂ (100%) and IL-1 β (94.5%) production by murine macrophages compared with the control ($p < 0,05$). Also, EOs did not affect NO production. *L. organoides* EO inhibited at least in 1.9 and 4-fold the mRNA expression of *COX2* and *IL6* genes. The western blot results demonstrated a concentration-dependent reduction in the phosphorylation of I κ B α , JNK and ERK, indicating that the anti-inflammatory effect induced by the *L. organoides* EO is associated with the interference in the activation of MAPK and NF- κ B signaling pathways. The GC-MS analysis identified carvacrol (58,6 %), thymol (9,3 %), trans β -caryophyllene (5,8 %), *p*-cimene (3,5 %) and γ -terpinene (1,3 %) as the major components of the EOs, which could be responsible of the anti-inflammatory activity of *L. organoides*, as previously reported for the genus^[2].

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Phytochemical profile and molecular characterization of *Hypericum vesiculosum*

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Introduction

Hypericum (Hypericaceae) genus includes about 500 species, but the most well-known species is *H. perforatum*, due to its antidepressant activity. Until today, most *Hypericum* taxa have not been yet studied for their phytochemical profile and biological properties, although they are used interchangeably. In Greece, 40 taxa of *Hypericum* genus are indigenous, and almost one third of them endemic.

Method

Individuals of *H. vesiculosum* Griseb. (section Drosocarpium), a subendemic Mediterranean species, were collected from Peloponnese (Greece). Fresh material was morphologically and anatomically studied. Dry material was extracted with methanol and analysed via LC-HRMS (ESI-qTOF) and LC-DAD-MS (ESI-quadrupole ion trap), and HPLC-DAD for the first time. Sequence analysis of nrITS region was also performed to genetically characterize the species.

Results / Discussion / Conclusion

Hypericum vesiculosum presents black glands on its flowers (as stripes), and occasionally on its leaves. Anatomically, its leaves are dorsiventral and hypostomatic, and the major part of its circular stem is filled with xylem. In the methanolic extract, over twenty compounds were identified, including chlorogenic acid, flavanols, quercetin glycosides, quercetin, biapigenin but also apigenin glucosides, naphthodianthrones and phloroglucinols. Sixteen major ingredients were quantified with a previously validated HPLC-DAD method. Flavonoids were the most abundant group of compounds (>100.0 mg g⁻¹ of dry extract). The concentrations of total phenolic acids and total naphthodianthrones were 9.3 and 7.2 mg g⁻¹, respectively, while phloroglucinols were below quantification limit. Moreover, sequence analysis of nrITS revealed a unique genetic profile of *H. vesiculosum*, which can be used for intra-generic discrimination and species identification.

Acknowledgments

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Formulation and *in vitro* antibacterial activity of *Maclura tinctoria* L., *Cassia fistula* L. and *Malachra alceifolia* L. against acne

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Introduction

Maclura tinctoria L., *Cassia fistula* L. and *Malachra alceifolia* L. [1] are used for treatment of the most common human diseases and health disorders in folk medicine of the population from the northern Colombian coast. Acne is one of the most common and chronic skin problem in most adolescents and young adults, *Propionibacterium acnes* and *Staphylococcus epidermidis* are the major bacteria found on skin causes acne [3].

Methods

In this study, chemical composition, formulation and antibacterial activity of the ethanol extract from cortex *M. tinctoria* L., *C. fistula* L. and leaves *M. alceifolia* L. were investigated. The chemical constituents of qualitative detection were examined by thin Layer Chromatography (TLC). The antibacterial activity was determined by agar gel diffusion and broth microdilution method against *Staphylococcus aureus* (ATCC 25923) y *Propionibacterium acnes* (ATCC 6919), the MIC was 62.5 to 1000 µg/mL, Kanamycin as a positive control, as a 1% DMSO negative control [2-4]. To the topic cream formulation O/W a statistical experimental design type Placket-Burman was developed, where seven factors (excipients) were evaluated [2,4].

Results/Discussion/Conclusion

The phytochemical analysis of the plants evaluated revealed the presence of flavonoids, cardiac glycosides, alkaloids, saponins, terpenoids, tannins and coumarins in the extract. The ethanol extract from *C. fistula* activity against *S. aureus* the range of inhibition zone between 7 to 15 mm and *M. alceifolia* against *P. acnes* 5 to 12 mm, ethanol extract of *M. tinctoria* have low inhibitory activity against bacterial strains of study. The MIC of potential two plant extracts which gave anti-bacterial activity against acne causing bacteria were determined using broth micro dilution method *C. fistula* activity against *S. aureus* 125 µg/mL and *M.*

acleifolia against *P. acnes* 62.5 µg/mL. These can also be an alternative and better option for resistant acne causing bacteria. The formulation cream obtained was: macrogol cetostearil-ether-12 at 1%, macrogol cetostearil-ether-20 at 3%, glycerin monostearate at 8%, stearyl alcohol at 8%, 5% vegetable oil, 5% dibutyl adipate, 10% glycerin, -alcohol benzyl, methylchloroisothiazolinone and methylisothiazolinone- 0.1%, butylhydroxytoluene 0.02%, extract *Cassia fistula* L. 7.5%, extract *Malachra alceifolia* L. 3.5% and water csp 100g, where the extracts showed persistent antibacterial activity.

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The efficacy of ketogenic diet in patients with moderate to severe psoriasis

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Introduction

Psoriasis is one of the most common chronic inflammatory skin disorders, affecting about 2% of the population (Armstrong *et al.*, 2017; Pona *et al.*, 2019). Several studies demonstrated that Weight loss (WL) could reduce psoriasis severity and inflammation (Gamret *et al.*, 2018; Ford *et al.*, 2018). Very low-calorie ketogenic diet (VLCKD) has been associated with a significant reduction in visceral adipose tissue and keton bodies have anti-inflammatory proprieties (Paoli *et al.*, 2013).

Method

The aim of this work was to evaluate the efficacy of an aggressive WL program with a ketogenic induction phase as first-line treatment of chronic plaque psoriasis. The program was conducted from May 2018 to October 2018 in hospital outpatient dermatology clinic.

The participants were adult overweight/obese patients (n=33) with stable chronic plaque psoriasis.

In particular a 8-weeks two-phase WL program consisting in a 4-weeks protein-sparing, VLCKD (oloproteic diet) (<500 kcal/day; 1.4 grams of protein/kg of ideal body weight/day of which approximately 50% by alimantar protein and 50% by whey protein supplement and aminoacids) and a 4-weeks LOGI (low glycemc and insulinemic diet) was performed. In order to improve the intestinal function and stress, the diet was integrated with medicinal plants tripahla, amalaki, haritaki, bibitaki and ashwagandha, respectively.

The primary endpoint was the reduction in the psoriasis area and severity index (PASI) at week 8. Main secondary endpoints included: improvement of PASI $\geq 50\%$ and $\geq 75\%$, reduction in body surface area (BSA) involved, improvement of ITCH severity (visual-analogue scale) and dermatology life quality index (DLQI) at week 8.

Results /Discussion /Conclusion

Dietary intervention with a mean body weight reduction of 11.5%, resulted in a significant reduction in PASI (mean \pm standard deviation, 11.5 \pm 5.4 [range, 7-29]): mean change, -10.6 [95% CI, -12.8 to -8.4] (p<0.001). A

reduction in PASI $\geq 50\%$ and $\geq 75\%$ was recorded in 32 (97.3%) and 21 (64.9%) patients, respectively. Treatment resulted also in a significant reduction of involved BSA and an improvement in ITCH severity and DLQI ($p < 0.001$). Moreover a significant reduction of inflammatory cytokines after treatments was observed. In untreated adult overweight patients with stable chronic plaque psoriasis an aggressive dietary WL program consisting in a very low-calorie ketogenic regimen followed by a balanced, hypocaloric mediterranean-like diet appeared an efficacious first-line strategy for improving disease severity. comparative studies with other dietary regimens and long-term efficacy data are warranted.

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Safety of low-carbohydrate diets: herbal and food supplements

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Eur Rev Med Pharmacol Sci. 2016 Jul;20(12):2613-21.

Very-low-calorie ketogenic diet with aminoacid supplement versus very low restricted-calorie diet for preserving muscle mass during weight loss: a pilot double-blind study

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Abstract

OBJECTIVE:

Obesity plays a relevant pathophysiological role in the development of health problems, arising as result of complex interaction of genetic, nutritional and metabolic factors. We conducted a dietary intervention case-control randomized trial, to compare the effectiveness on body composition of two nutritional protocols: a very-low-carbohydrate ketogenic diet (VLCKD), integrated by an aminoacid supplement with whey protein, and very low restricted-calorie diet (VLCD).

PATIENTS AND METHODS:

The clinical study was conducted with a randomized case-control in which twenty-five healthy subjects gave informed consent to participate in the interventional study and were evaluated for their health and nutritional status, by anthropometric, and body composition evaluation.

RESULTS:

The results of this pilot study show that a diet low in carbohydrates, associated with a decreased caloric intake, is effective in weight loss. After VLCKD, versus VLCD, no significant differences in body lean of the trunk, body lean distribution (android and gynoid), total body lean were observed ($p > 0.05$). After VLCKD, no increasing of sarcopenia frequency, according ASSMI, was observed.

DISCUSSION:

Many studies have shown the effectiveness of the ketogenic diet on weight loss; even if not know how to work effectively, as some researchers believe that the weight loss is due to reduced calorie intake, satiety could also be induced by the effect of the proteins, rather than the low-carbohydrates.

CONCLUSIONS:

Our pilot study showed that a VLCKD was highly effective in terms of body weight reduction without to induce lean body mass loss, preventing the risk of sarcopenia. Further clinical trials are needed on a larger



population and long-term body weight maintenance and risk factors management effects of VLCKD. There is no doubt, however, that a proper dietary approach would impact significantly on the reduction of public expenditure costs, in view of prospective data on increasing the percentage of obese people in our nation.

The oxidative stress index in human population with arterial hypertension and diabetes mellitus

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Introduction

A clinical study was conducted in a cohort of human subjects from 60 to 75 years of age to assess the correlation between disease diagnosis and oxidative stress damage in diabetes mellitus (DM) and arterial hypertension (AHT) to provide a sound basis to design a clinical protocol for antioxidant adjuvant therapy for DM and AHT.

Method

The values of the oxidative stress index (OSI) were determined from the experimental values of biomarkers in erythrocyte lysates obtained from human blood samples including total antioxidant levels, specific biomarkers of oxidative damage, and antioxidant enzyme activities. Three study groups were formed: Group I included DM patients (110 subjects), Group II included AHT patients (112 subjects); and the control group included healthy volunteers (123 subjects). Inclusion criteria were as follows: i) patients of both sexes from 60 to 75 years of age with a confirmed diagnosis of DM (types I and II) and/or AHT (degrees I, II, and III) from Santo Domingo Province, ii) patients with disease evolution of 2 years or more, iii) nonsmokers, iv) patients who did not regularly consume alcoholic drinks or stupeficient drugs, and v) patients who did not consume antioxidants during the last 12 months. Exclusion criteria were as follows: i) other chronic degenerative diseases, and ii) participation in other clinical studies. The data of all groups were analyzed using SPSS 9.0 software. A nonparametric Friedman test was used to compare several related subgroups, and changes within the groups and subgroups were tested using the Wilcoxon paired test. A Mann-Whitney U test was used to estimate significant differences ($p < 0.05$) between the subgroups.

Results & Discussion

The results indicated that age, sex, and ethnicity did not influence the OSI values. Severe oxidative stress was observed in subgroup IIc (AHT III), moderate oxidative stress was observed in Subgroups Ib (DM II)

and IIb (AHT II), mild oxidative stress was observed in Subgroup Ia (DM I), and no oxidative stress was observed in Subgroup IIa (AHT I).

Conclusion

The results support the design of clinical trial protocols for adjuvant antioxidant therapy to increase the efficacy of standard therapies for AHT II and III and for DM II. Antioxidant therapies for DM I and AHT I are not recommended due to the presence of only mild oxidative stress or no oxidative stress, respectively.

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Mitochondrial dysfunction and fibromyalgia: possible pathophysiological mechanisms of aglucidic nutritional therapy in fibromyalgic treatment.

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Introduction

Fibromyalgia (FM) is a worldwide diffuse musculoskeletal chronic pain condition that affect approximately 5% of the population worldwide (1).

Fibromyalgic patients commonly suffer from chronic overlapping symptoms including muscle-tendon pain, chronic fatigue syndrome, not restored sleep, loss of memory, concentration difficulties, depression, anxiety, headache and intestinal illnesses like disbiosis (Small intestine bacterial overgrowth SIBO), irritable bowel syndrome (IBS), leaky gut and meteorism which are thought to be related to central sensitization (2,3).

Although the etiology is unknown it has been supposed a possible relationship between mitochondrial dysfunction, oxidative stress, and inflammation in FM (4).

Specially it is hypothesized that reactive oxygen species (ROS), caused by oxidative and nitrosative stress, by inhibiting mitochondrial function can be involved in muscle pain and central sensitization as typically seen in these patients (5). In addition to reactive oxygen species formation, genetic factors, aging, and reduced mitochondrial biogenesis all contribute to mitochondrial dysfunction (6). If mitochondrial dysfunction is also present in central neural cells, this could result in lowered ATP pools in neural cells, leading to generalized hypersensitivity and chronic widespread pain (7). Neuroinflammation in fibromyalgia is multifactorial and supposedly correlated to pain and stress. However, attribution of neuroinflammation and central sensitization to a primary neurogenic origin is premature without integrating the coexistence of intestine bacterial and fungal overgrowth, vitamin D deficiency, and mitochondrial dysfunction (8).

Therefore, targeting fungal and bacterial overgrowth, increased ROS and mitochondrial dysfunction could offer a solution for the chronic pain in these patients. In this context, the positive effect of the aglucidic nutritional therapy is probably due to multiple mechanism such as the modulation of gut microbiota by inhibiting the glucose depending, pathogenic strains proliferation combined with several mechanism that together reduce neuronal excitability. Moreover the aglucidic nutritional therapy could improve the



mitochondrial function because ketone body elicits compensatory mechanism acting to augment mitochondrial mass and bioenergetics via the PGC1 α -SIRT3-UCP 2 axis (9).

Method

33 patients were underwent a 28-day integrated carbohydrate-free diet (<10 g carbohydrates, 1.4 g/IW protein, 10 g short chain fatty acids (MCT) and 20 g extra virgin olive oil) with elimination of excitotoxins and gluten adding an antioxidant therapy for improving mitochondrial performance.

The diagnostic criteria chosen for the clinical evaluation at time 0 and after 28 days of the fibromyalgic patient were two: The Scale of Pain, the Widespread Pain Index (WPI), and the Symptom Severity Scale (SS-Score).

These criteria evaluate the painful areas reported by the patient on body source (WPI) and the associated symptoms (SS-Score).

Results and Conclusion

After the treatment, the patients, had a reduction of tender points demonstrated by a reduction of the value of WPI from 9,2 to 3,4.

Moreover patients experienced an improvement of symptoms such as intestinal illness and a reduction or disappearance of meteorism and of gastroesophageal reflux, an improvement of quality and quantity of sleep, of restored sleep and a reduction or absence of fatigue and better memory and concentration. The SS-score in fact it is gone from 15,5 to 5,3.

In conclusion, this study demonstrated not only the efficacy of aglucidic nutritional therapy in solving the symptomatology of fibromyalgia, but also the therapeutic action of supplementation and antioxidant intervention which implements further the therapeutic efficacy of diet.

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Effect of pilates training on people with fibromyalgia syndrome

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Abstract

Pilates is a particular exercise approach that was founded on the teachings of Joseph Pilates and was initially practiced almost exclusively by athletes and dancers. Pilates has become a fast-growing, popular trend in rehabilitation and fitness programs in recent years^{1, 2}. Pilates can be described as a method combining Oriental and Western philosophies including yoga, dance, durability-strength training, and gymnastics.

The goal of Pilates training is improvement of general body flexibility and health, emphasizing core (truncal) strength, posture, and coordination of breathing with movement. Although Pilates exercise is generally adopted in training programs for healthy people as part of general fitness programs, it has been suggested as a therapeutic modality for several musculoskeletal disorders. Fibromyalgia syndrome, a chronic condition typically characterized by widespread pain, nonrestorative sleep, fatigue, cognitive dysfunction, and other somatic symptoms, negatively impacts physical and emotional function and reduces quality of life. Exercise is commonly recommended in the management of people with fibromyalgia, and interest in examining exercise benefits for those with the syndrome has grown substantially over the past 25 years. Treatment of FMS is usually symptomatic because of the lack of understanding of its etiology and pathophysiology, and several treatment modalities ranging from antidepressant therapy to biofeedback and electroacupuncture have been suggested³⁻⁵. A range of pharmacologic and nonpharmacologic management options have also been examined in evidence-based guidelines and reviews⁶. A stepwise program emphasizing education, certain medications, exercise, cognitive therapy, or a combination of all these modalities has been recommended in the light of current evidence⁵. Exercise programs were reported to be helpful in FMS patients in several studies, and the programs including stretching, strength maintenance, and aerobic conditioning were accepted as a standard treatment protocol⁷⁻¹². A recent study has suggested the beneficial effect of supervised aerobic exercise training and strength training on physical capacity and clinical symptoms in FMS patients¹³. In this communication authors reported some scientific evidences on the effects of Pilates on pain, functional status, and quality of life in fibromyalgia, which is known to be a chronic musculoskeletal disorder.

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Antioxidant Activity in weaned piglets by standardized water-soluble extract of *Wendtia calycina* Leaves

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In pig nutrition the use of herbal extracts diet supplementation has shown benefit effects in some physiological parameters like growth performance, feed efficiency and immune-related blood characteristics. Likewise, a few herbal extracts can stimulate nutritional mechanisms including secretion of digestive enzymes and feed intake, possibly through of immune stimulation, anti-bacterial, anti-viral and antioxidant properties. *Wendtia calycina* (Griseb.), Vivianiaceae, is a Paraguayan herbaceous plant commonly known as burrito. From *W. calycina* leaves, a standardized, water soluble extract (WSE) rich in phenylpropanoid glycosides has been developed on an industrial scale to be used as a food supplement, cosmetic, phytomedicine, and ingredient of different formulations. The extract is prepared by a standardized procedure that includes ultrasonic extraction with aqueous EtOH (60% v/v), followed by a spraydrying using maltodextrin as excipient. WSE represents a concentrate of phenolic compounds and its main constituents are phenylpropanoid glycosides (PPGs), principally verbascoside (7.5 %) and isoverbascoside (1.1 %). This product also contains various benzoic acid derivatives as minor constituents. Our previous study indicated that burrito leaves are a very good source of phenylpropanoid glycosides, principally verbascoside. From *W. calycina* leaves, a standardized, water-soluble extract rich in phenylpropanoid glycosides (WSE) has been developed on an industrial scale to be used as a food supplement, cosmetic, phytomedicine, and ingredient of different formulations.

In this communication study, we investigated the *in vitro* overall antiradical efficiencies of the WSE and the *in vivo* effects on growth performances and blood oxidative status in weaned piglets supplemented with WSE. Piglets (168) were randomly selected and assigned to 1 of 2 dietary groups (WSE = 0 and 5 mg/kg of feed). Growth performances of piglets were not affected by dietary supplementation. Total antioxidant activity of whole blood tended to be higher in piglets fed diet supplemented with WSE.

Literature

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Chemical composition and antioxidant activity of algerian propolis

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Propolis is a resinous hive product actually marketed by the pharmaceutical industry and [health food stores](#) for its claimed beneficial and preventive effects on [humanhealth](#). Propolis shows a composition extremely variable and the vegetation at the site of collection determines its chemical diversity (Salatino et al., 2011). This characteristic determines a broad spectrum of biological activities and highlights the high significance of chemical standardization to connect a particular chemical propolis type to a specific type of biological activity (Bankova, 2005).

This communication describes chemical composition and antioxidant activity of fourteen Algerian propolis (AP) samples collected in different regions of North Algeria. The characterization by HPLC-DAD of chemical profiles of AP samples allowed the identification of two main types directly related to their constituents. The main secondary metabolites of the representative two type of Algerian propolis (AP9 and AP4) were identified by preparative chromatographic methods and NMR and MS elucidation. Four caffeate esters and six flavonoids characterized the AP9 type, whereas seven labdane and two clerodane diterpenes, together to one polymethoxyflavonol, are the main constituents of AP4 type. Subsequently, two specific HPLC-MS/MS methods for detection of AP9 and AP4 marker compounds were developed to study the chemical composition of AP. All samples showed a chemical profile of AP9 and/or AP4 types suggesting a characteristic chemical composition for propolis from North Algeria. The chemical data agree with antioxidant activity evaluated by DPPH° assay (Mencherini et al., 2007). The samples containing exclusively the AP4 markers don't showed free radical scavenging activity ($SC_{50} > 100 \mu\text{g mL}^{-1}$) while significant activities ($SC_{50} 32\text{-}82 \mu\text{g mL}^{-1}$) were observed for those with AP9 profile. For the latter the antioxidant activity is directly correlated to presence of caffeate esters and flavonoids, well-known antioxidant natural compounds (Kumazawa et al., 2004).

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Collagen Ultrasound. A new diagnostic technique

Monaco

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Collagen is the most abundant protein in the human body (25% of the protein mass):

Skin and subcutaneously 72%; Cartilage 50%; Bones 23%.

The sonographic study of collagen is performed at a cutaneous and articular level.

Skin:

The papillary dermis underlying the epidermis consists of lasso and less dense connective tissue and has many collagen fibers and few elastic fibers. It is rich in blood vessels and nerve endings and has more cells than the reticular one. It rises in ledges called dermal papillae. The reticular dermis extends to the hypodermis. It is made up of many robust collagen and elastic fibres that follow the same direction. It can be damaged by excessive distension (accumulation of fat or during pregnancy), resulting in the formation of a whitish anti-aesthetic scar, the stretch mark.

Sonographic assessment of collagen related to dermis:

Ecographically we can indicate three well defined skin layers:

- the hyperechogenic line of the epidermis;
- the hypoechogenic line of the papillary dermis;
- the finely unhomogeneous, hyperechogenic ecostructure of the reticular dermis.

In relation to the modification of the dermis proteins (collagen and elastin), responsible for the loss of skin tone, ultrasonography analyses:

- the thickness of the dermis (3-4 mm);
- the echogenicity of the dermis which is reduced with the age of senile;
- the SLEB (Subepidermal low echogenic band), i.e. the hypoechogenic line at the superficial dermis level (papillary dermis) due to the elastosis process, i.e. the basophilic degradation of collagen. In the ultrasound evaluation in addition to the qualitative evaluation of the dermis, supplemented by elastosonography, a



quantitative evaluation is also carried out (measurement of SLEB and epidermis-derma distance) necessary for the evaluation in the therapeutic path as reference parameters-response to medical treatment stages.

Articular cartilage:

The main component of the articular surface is a special tissue called "cartilage ialina". When this tissue is damaged, the articular surface is no longer smooth and the movement of the bones on a hard and damaged articular surface becomes difficult and causes pain. The cartilage damaged over time also can lead to osteoarthritis i.e. the painful degenerative wear of the whole joint.

Ultrasound evaluation integrated by the elastosonographic study:

is performed at the knee level. Hyaline articular cartilage adjacent to the subchondral bone appears as an anechogenic band on ultrasound. The normal thickness is mm 2-3 mm. The hyaline cartilage, the subchondral bone surface and the sub-chondral bone should be considered with a single anatomic unit. Hyaline cartilage is characterised by high elasticity which is reduced with age and pathological processes. In relation to the elasticity score, we have defined 4 degrees of elasticity by elastosonography:

Score 0 -1 regular elasticity; Score 2 reduced elasticity; Score 3 substantial rigidity.

The cutaneous and articular ultrasound examination of the suffering of collagen can be considered a reliable methodical, not strictly dependent operator (thanks to the integration ultrasound + elastosonography), low cost and non-damaging (does not expose to radiation).

Conclusion

Ultrasonography allows to evaluate the physiological/pathological state of collagen and in particular:

- Confirm the clinical suspicion
- Define the degree of disease
- Evaluate the response to therapy.

Protein and Amino Acid Integration in the Sarcopenic Patient

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Introduction

Sarcopenia is a pathological condition of muscles, characterized by the diminution of volume and muscular strength, and it is associated to an increased risk of functional decline, fragility, hospitalization and death. The incidence of sarcopenia increases with age and takes to high individual, social and health costs. Finding adequate nutritional measures to maintain muscle health, preserving function and independence for the growing elderly population would have important scientific and social implications.

Method

Resistance exercise and diet are essential critical factors for preserving muscle health as we age. Among the nutrients that humans take, amino acids directly activate the synthesis of muscle proteins. The essential amino acid leucine, in particular, functions as a stimulation signal .A supplement based on hydrolyzed collagen of porcine origin easily used by man for optimal histocompatibility, organic silicon and vitamin C (which contribute to the normal formation of collagen), MSM, Methyl Sulfonyl Methane, (useful for elasticizing collagen molecules) , OPC, ProCyanidine Oligomers, and Vitamin E (useful for slowing its degradation), is proposed to reduce the tendency to sarcopenia. The body produces its own collagen every day, but as production decreases with age, the available amount of collagen quickly becomes insufficient and the different parts of the body gradually deteriorate. The integration of whey protein, special amino acids, bioavailable silicon, and MSM allow to respect the turnover of collagen and therefore to protect the skin and joints. Hydroxyproline, which is critical for collagen stability, is synthesized by hydroxylation of the amino acid proline; the reaction requires organic silicon and vitamin C to allow the addition of oxygen.

Results / Discussion / Conclusion

The long-term intake of leucine-enriched essential amino acids has a synergistic effect with physical exercise to increase muscle mass, strength and walking speed in elderly individuals and can be an effective countermeasure against sarcopenia. When designing an "optimal" diet for the prevention of sarcopenia, the optimal intake of protein for muscle health in the elderly must be taken into account. While proteins, essential amino acids (EAA) and leucine (mainly in combination with exercise) have been recognized as established factors, it is predicted that the intake of collagen, added to other substances, is useful to reduce the tendency to sarcopenia . Collagen is a ubiquitous protein, the most represented in the body, a fundamental constituent of structures such as muscles, cartilages, bones, etc.



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Antidiabetic plants in traditional Lebanese medicine

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Introduction

Diabetes affects 425 million people in the world and more than 39 million people in the Middle East and North Africa region. In Lebanon, 585.400 cases were recorded in 2017, equal to 14.6% of the adult population. The major Lebanese ethnic groups have a rich ethnomedicinal tradition. In some rural regions, the use of plants for the treatment of diseases associated with physiological disorder such as diabetes is very common. Therefore, the present work documented this precious knowledge as well as the experience of the traditional healers.

Method

Regular field trips to different areas of the twenty-six districts “aqdya or qadaa” of the six governorates “mohaafazah” of Lebanon were conducted to collect ethnomedical information and herbarium specimens. The rural population, herbalists and village elders were interviewed in addition to the traditional healers of each community approached thanks to the help of the socio-cultural organizations of the different ethnic groups.

Results / Discussion / Conclusion

In total, 111 plants belonging to 37 families (Asteraceae, Lamiaceae, Rosaceae, Fabaceae and Apiaceae mainly) were recorded as remedies against diabetes and hyperglycemia. Among them, 73 species are indigenous and 8 endemic. Fifteen plants (e.g., *Origanum syriacum* L., *Salvia fruticosa* Mill., *Cynara scolymus* L., *Rosmarinus officinalis* L., *Ceratonia siliqua* L. and *Rheum ribes* L.) were frequently mentioned and highly recommended by both the traditional healers and herbalists. The plant parts most commonly used were leaves and flowers (28.8% and 20.1%, respectively), followed by fruits (15.1%) and seeds (10.1%).

The most frequent preparations were decoction (50%) and infusion (30.1%), exclusively for internal administration.

Preliminary studies have been carried out on some ethnomedicinal plants such as *Allium* spp. (Alam Khan, 2011), *Atriplex halimus* L. (Chikhi et al., 2014), *C. siliqua* (Gruendel et al., 2007) and *Juglans regia* L. (Asgary et al., 2008), used in Lebanon for their hypoglycemic effects. These antidiabetic plants exert their action by different mechanisms stimulating β -cells function and number, thus increasing insulin release or enhancing glucose-6-phosphatase and fructose 1,6-biphosphatase enzyme activities, slowing the absorption of carbohydrates and the inhibition of glucose transport (Afifi-Yazar, 2011). However, the plant products may interact with the conventional antidiabetes drugs and a prudent approach should be adopted before their administration. In any case, the collected ethnobotanical information is important for the selection of plant material to be subjected to pharmacological research leading to the discovery of new drugs.

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Anti-diabetic effects of α -amyrin and lupeol produced by dual PPAR δ / γ agonist action and AMPK α 1 activity

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Introduction

α -amyrin and lupeol, are triterpenes found in a wide variety of medicinal plants. These triterpenes have been shown to have beneficial effects on health, among their properties are anti-cancer [1], anti-inflammatory [2] and anti-diabetic effects [3]. Within the anti-diabetic effects, the molecular mechanism by which they exert said effects has not been elucidated, being of pharmacological importance for new alternatives in the treatment of type 2 diabetes (DT2). AMPK α 1 is considered as a regulator of energy homeostasis, which together with PPARs play an important role in the metabolism of lipids and glucose, regulating the expression of genes involved in metabolism. AMPK α 1 [4], PPAR δ and PPAR γ , are considered therapeutic targets in the treatment of T2D and other metabolic diseases, which may explain the anti-diabetic effects of α -amyrin and lupeol. AMPK α 1 and PPAR δ participate mainly in the incorporation and catabolism of lipids. PPAR δ promotes the expression of the fatty acid transporter FATP among others associated with β -oxidation, while PPAR γ regulates GLUT4 expression which transports glucose to the interior of the cell and participate in adipogenic process. The aim of this research is to evaluate if these triterpenes have an effect on glycemia in vivo and lipid accumulation in vitro, also AMPK α 1, PPAR δ and PPAR γ participation in these effects.

Method

Evaluation of α -amyrin and lupeol on:

- Glycemia in normal mice by oral glucose tolerance test (OGTT).
- Lipid accumulation in adipocytes 3T3-L1 (Oil Red staining).



-Genes Expression and proteins PPAR δ , PPAR γ , FATP and GLUT4 in myoblasts C2C12 by RT-qPCR and Western blot respectively.

-Activation of AMPK α 1 by Western blot.

-GLUT4 by immunohistochemistry in myoblasts C2C12.

Results / Discussion / Conclusion

α -amyrin and lupeol decreases glycaemia in mice and lipid accumulation in adipocytes, and increase the mRNA expression and proteins of PPAR δ , PPAR γ , FATP and GLUT4 in C2C12 myoblasts. These results show a possible dual mechanism of action of these compounds, activating dual PPAR δ / γ and AMPK α 1 improving a balance of glucose and lipid metabolism. This work provides important information in the field of pharmacology, for the subsequent development of new and better drugs of natural origin with multimodal effects, which avoid the recurrent polypharmacy in diseases as DT2. The new therapeutic strategies could be focus on decrease of glycemia and lipid accumulation, and that eventually reverse the metabolic imbalance present in T2D and other diseases associated with the metabolic syndrome.

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Very-low-calorie ketogenic diet with aminoacid supplement versus very low restricted-calorie diet for preserving muscle mass during weight loss: a pilot double-blind study

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Abstract

OBJECTIVE:

Obesity plays a relevant pathophysiological role in the development of health problems, arising as result of complex interaction of genetic, nutritional and metabolic factors. We conducted a dietary intervention case-control randomized trial, to compare the effectiveness on body composition of two nutritional protocols: a very-low-carbohydrate ketogenic diet (VLCKD), integrated by an aminoacid supplement with whey protein, and very low restricted-calorie diet (VLCD).

PATIENTS AND METHODS:

The clinical study was conducted with a randomized case-control in which twenty-five healthy subjects gave informed consent to participate in the interventional study and were evaluated for their health and nutritional status, by anthropometric, and body composition evaluation.

RESULTS:

The results of this pilot study show that a diet low in carbohydrates, associated with a decreased caloric intake, is effective in weight loss. After VLCKD, versus VLCD, no significant differences in body lean of the trunk, body lean distribution (android and gynoid), total body lean were observed ($p > 0.05$). After VLCKD, no increasing of sarcopenia frequency, according ASSMI, was observed.

DISCUSSION:

Many studies have shown the effectiveness of the ketogenic diet on weight loss; even if not know how to work effectively, as some researchers believe that the weight loss is due to reduced calorie intake, satiety could also be induced by the effect of the proteins, rather than the low-carbohydrates.

CONCLUSIONS:

Our pilot study showed that a VLCKD was highly effective in terms of body weight reduction without to induce lean body mass loss, preventing the risk of sarcopenia. Further clinical trials are needed on a larger

population and long-term body weight maintenance and risk factors management effects of VLCKD. There is no doubt, however, that a proper dietary approach would impact significantly on the reduction of public expenditure costs, in view of prospective data on increasing the percentage of obese people in our nation.

Ketogenic diet and menopause: today and future

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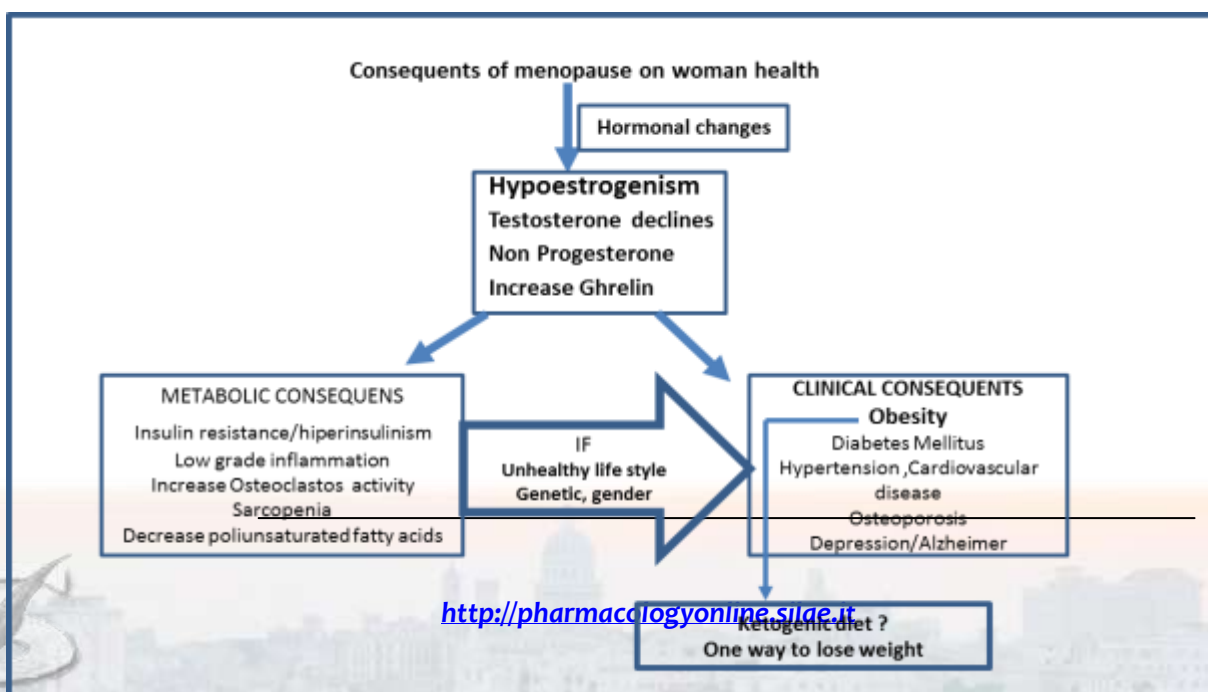
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Lecture

During climacteric period reproductive hormones were on a speedy decline, causing new symptoms called Climacteric syndrome. Estrogens declination cause: a shift in body fat from hips and thighs to abdomen, a rise in insulin resistance, which causes weight gain, impaired production of leptin and neuropeptide Y (hormones that help regulate appetite and weight balance). **Testosterone declines**, leading to a loss of muscle mass (that contribute to insulin resistance) and, as a result, a slower glucose metabolism and compensatory hyperinsulinism. **Progesterone declines is associate with** an increase in anxiety and depression. **Ghrelin** (the hunger hormone) **increases** causing: a dramatic increase in hunger and cravings, and finally, in response to these significant hormonal shifts, gut microbiome goes through a massive shift as well (1-4).

All this physiologic changes may contribute to development of metabolic (Diabetes Mellitus, dyslipidemias), cardiovascular diseases (Coronary artery disease, arterial hypertension), mental disorders (depression, Alzheimer disease), and osteoporosis all of the have obesity (adiposity) as a risk factor, for these reason weight-loss therapy is an important way for prevention of all these above mentioned.(4)



Analysis and in vivo evaluation of physio-balancing and soothing effect of emulsions containing ALOE ARBORESCENS GEL

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Introduction

The aloe gel is obtained from the central part of fresh leaves. The leaves are drained to remove the juice rich in anthracene derivatives, after they are opened with a longitudinal cut and the gelatinous substance is removed from the central part (Aloe gel). It is collected and the solution is added with preservatives such as sodium benzoate and potassium sorbate (0.3% according to Agarwal (1997)). The resulting gel can be used in different ways, from food to cosmetics. Aloe Arborescens has different properties. The topical application of the product has a moisturizing, protective, insulating and emollient function thanks to the high water content [1].

Method

Our research included in a first time the analysis of aloin, that cannot exceed the concentration of 0.1 ml / L (EC directive 1998) in ALOE ARBORESCENS GEL 1:1 samples and then the formulation of cosmetic creams that have been evaluated *in vivo* for their moisturizing effect.

Aloe Arborescens leaf juice was produced in the South of Italy, the HPLC analyzes were carried out on different batches of gel obtained according to a GMP manual (Good Manufacturing Standards). We used ALOE ARBORESCENS GEL 1:1 as active component in emulsions O/W and we verified the moisturizing action *in vivo* by the measurement of TEWL and corneometry on 20 volunteers aged between 20 and 65 years. At the beginning (T0), after 1 hour and 24 hours (T1h, T24h) the following instrumental evaluations were carried out: 1) Measurement of TEWL (Trans-epidermal water loss) using the Tewameter DUAL MPA 580 (Courage & Khazaka) probe, capable of measuring transcutaneous water loss. A significant reduction in the TEWL value indicates an increase in the functionality of the skin barrier and therefore a saving of water from the skin layers. 2) Measurement of the corneometry using the Corneometer® DUAL MPA 580 (Courage & Khazaka) probe, capable of quantizing the water content of the stratum corneum [2]. The study

proceeded with the induction of an erythema on the fly part of the arm by pure Sodium Laureth Sulfate (equal to a concentration of 30%) and rinse with water gently. The erythema produced, was measured by a Mexameter® MX 18 probe (Courage+Khazaka electronic GmbH). Mexameter® is a probe capable of measuring, through reflectance spectrophotometry, the two main components responsible for skin color: melanin and hemoglobin, indicating the value of the erythema index [3]. Just after the measurement on two test sites, indicated as T0', 0.5±0.10 g of cream and placebo on the test sites were applied and after 30 minutes (T30 ') the erythema index was re-measured to evaluate the soothing power of the tested emulsions.

Results

We verified that emulsion containing a concentration of 0.5 or 1% (w/w) of Aloe Gel was able to moisturize the skin after 1 hour and 24h respect a placebo, moreover the emulsions containing Aloe gel were able to decrease significantly erythema compared to placebo. All data were statistically treated by Student t and considered reliable when the statistical probability was $p < 0.05$.

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Olive tree cultivation waste recycling

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The growing human population and urbanization led an increase in agricultural waste, whose management practices differ for developed and developing nations and for urban and rural areas. Decrease total waste by recycling of refuse, by extracting phytochemicals or organic molecules to use as functional material or energy carrier are some methods used to reduce the problem of their disposal. During the harvesting and fabrication process of olive fruits, twenty-five kilograms of twigs and leaves per tree and 30 million m³ of olive mill wastewater are produced annually in the Mediterranean area. The olive processing wastewater is not easily biodegradable and needs to be detoxified before it can properly be used in agricultural and other industrial processes. In this work phenolic content and antioxidant value of pomace oil, wastewater and olive tree leaves were evaluated to offer sanitary living conditions to decrease the amount of waste that arrives or exit from the society and encourage their reuse within the society. Moreover, the nutraceutical properties of an herbal tea, made from olive leaves, has been evaluated.

Working with Indigenous Biocultural Knowledge in Natural Resource Management

Gerry Turpin, Australia

For thousands of years Australian Indigenous peoples have lived on and managed this country, shaping the landscapes and environments, and adapting to changing climates. The knowledge that has been accumulated through intimate and sustained connections to the land had been passed down orally through generations, existing in stories, paintings, song and dance. While a lot of knowledge has been lost due to colonisation and in some places resulted in disconnection to country, many Indigenous groups continue to practice culture.

A more recent term recognising this knowledge is Indigenous Biocultural Knowledge (IBK). IBK is 'knowledge that encompasses people, language and culture, and their relationship to the environment' (Ens et al, 2014). Globally, IBK is well recognised, accepted and respected in contemporary biodiversity conservation and management. However, in Australia, where Indigenous people manage nearly half of the continent, there is a lack of understanding and reluctance to recognise IBK and explore ways that it can be respectfully employed with Western Science to benefit communities and society. This strong and diverse presence on country presents a great opportunity to work with Indigenous knowledge systems and strengthen awareness of biocultural knowledge.

This presentation will highlight the view that IBK has an important role in rapid and uncertain times of environmental changes; demonstrate some of the potential opportunities; and also some important considerations in cross-cultural research.

Evaluación de los perfiles químicos de 7 variedades de aguacate (*Persea americana*) y su efecto sobre el hongo fitopatógeno *Colletotrichum spp.*

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Resumen

Los cultivos de aguacates (*Persea americana*), han ganado gran importancia en la economía, debido a su alta demanda comercial a nivel mundial. La principal forma de protección es mediante el uso de fungicidas de origen sintético, los cuales generan perjuicios al medio ambiente y riesgos a la salud (Kim, Kabir, and Jahan 2017), además pueden estar involucrados en la resistencia microbiana (Gressel 2018). Entre las enfermedades que más afectan los cultivos, en campo y postcosecha, se encuentra la Antracnosis, causada por hongos del género *Colletotrichum*, que atacan frutos y hojas, siendo la mayor causa de las pérdidas de cosechas (Hernández-Lauzardo et al. 2015).

En la búsqueda de nuevas alternativas para el control de fitopatógenos se han empleado plantas resistentes que disminuyen la colonización y la severidad de las lesiones, así como también, el aprovechamiento de los mecanismos de defensa propios de la planta, mediante la aplicación de compuestos que estimulan la producción de metabolitos como las fitoalexinas (Paxton 1981).

El objetivo de este estudio fue determinar el perfil químico de los extractos metanólicos de hojas de 7 variedades de aguacate (Hass, Fuerte, Reed, Colin Reed, Nabal y Choquette) y establecer su actividad inhibitoria sobre el crecimiento del hongo *Colletotrichum spp.*

Las hojas de las diferentes variedades fueron extraídas con metanol, el sobrenadante fue filtrado y concentrado. Posteriormente se hicieron los perfiles químicos de los extractos mediante técnicas cromatograficas (HPLC-DAD); los ensayos de inhibición del crecimiento radial del hongo fueron realizados en PDA suplementado con los extractos a las concentraciones de 10, 100 y 1000 ppm. Este fue expuesto a los diferentes extractos incubado a 24 °C con fotoperíodo de 12 h y seguimiento diario por 8 días.

Los extractos de las variedades de hojas de aguacate que más presenta inhibición del hongo *Colletotrichum spp.* fueron Hass, Fuerte, Reed y Colin Reed a 1000 ppm con aproximadamente 25%. La variedad Fuerte se destacó por presentar inhibición del crecimiento del hongo a todas las concentraciones evaluadas. Este

estudio se emplea como base para búsqueda variedades resistentes y el diseño moléculas fúngicas más activas y específicas.

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Bio-guided fractionation to assess the antiproliferative activity of *Hymenaea courbaril* L. leaves on pc-3 prostate cancer cells

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Introduction

Cancer is the first cause of non-violent death worldwide and has become a public health issue. Prostate cancer has the highest incidence wide-reaching and it is the first cancer type with the highest prevalence within one to five years (Globocan, 2018). Chemotherapy, one of the most frequent treatments, which success reside on the tumor's sensitivity to chemical, natural and synthetic agents. However, tumoral cells display side effects to conventional anti-proliferative drugs, therefore it becomes necessary the search for new effective and safer molecules. The stinking toe (*Hymenaea courbaril* L.) is used in South America's traditional medicine to treat a large number of diseases (Boniface et al., 2017). Ethnobotanical, ethnomedicinal, and phytochemical properties are known but its therapeutic scope has not yet been scientifically proven.

Methods

Leave extracts of different polarities followed by bioassay-guided fractionation are used to determinate the cytotoxicity on androgen-independent prostate cancer cells, PC-3 and the selectivity index on a control healthy cell line, MRC-5. The extraction was carried out by the Soxhlet method obtaining Hexane, Dichloromethane, and Ethanol total extracts. These were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, followed by 3 fractionations, the subsequent activities were studied on the resulting active fraction: MTT assay, Gas Chromatography-Mass Spectrometry (GC/MS) and immunofluorescence. This, to the determinate the majority compound, and again the cytotoxic activity, and the immunofluorescence to reveal the morphological changes of the cells when are faced with the majority compound and the positive control.

Results / Discussion / Conclusion

The best cytotoxic activity was obtained from the hexane extract with an $IC_{50}=107.56 \mu\text{g/mL}$, leading it to its first fractionation, resulting in 20 fractions, then the fraction 2 presented the best cytotoxicity with an $IC_{50} =$

42.53 $\mu\text{g}/\text{mL}$. At that point, 3 sub-fractions were also obtained by column chromatography (A, B, C subfractions), and cytotoxicity was determined once more against the PC-3 cell line. The 2B subfraction obtained an $\text{IC}_{50}=80,41 \mu\text{g}/\text{mL}$, leading it to the last fractionation on an Rp18 column chromatography resulting in 6 more fractions, the 2B.3 fraction was selected by its cytotoxicity $\text{IC}_{50}=86,81 \mu\text{g}/\text{mL}$ and selectivity index, low damage on the healthy cells $\text{IS}= 1,71$. By the GC/MS analysis, it was determined that the majority compound within the fraction was Caryophyllene oxide (24,95%), which has an $\text{IC}_{50}=45,7 \mu\text{g}/\text{mL}$. When the hexane extract is faced to the cells, the microtubules are strongly affected, in addition, the cells have a considerable increase in size, variable morphologies, the nuclear content is found outside the envelope nuclear, typical characteristics of the induction of apoptosis are appreciated like the positive control Paclitaxel which destabilizes microtubules, increases cell size, induce the appearance of apoptotic bodies and different nuclear morphologies typical of the mitotic catastrophe (Mc Gee, 2015). These outcomes demonstrate that leave extracts, fractions and especially the majority compound, Caryophyllene oxide of *Hymenaea courbaril* L. can be used as potent and selective anti-proliferative agents, particularly because they show an acceptable safety profile, which has become necessary for the urgent search for more effective and safe medicines.

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Estudio químico y actividad antitumoral de *Punica granatum* Lam

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Introducción

Punica granatum L. es una planta medicinal empleada tradicionalmente, a nivel internacional, para el tratamiento del cáncer de próstata¹, cáncer de colón² y en enfermedades asociadas a la diabetes mellitus³⁻⁴. Sin embargo, a pesar del amplio uso que tiene en la población los estudios científicos sobre la especie, en Cuba, son escasos. En este estudio se determinaron los parámetros de calidad del fruto de *Punica granatum* L., se realizó la caracterización de los extractos hidroalcohólico y seco, se aislaron e identificaron los compuestos mayoritarios del extracto seco y se evaluó su actividad antitumoral.

Método

Los parámetros de calidad de la droga fueron evaluados siguiendo los procedimientos establecidos por la OMS (1992)⁵. Los extractos fueron caracterizados mediante sus perfiles CCD, CLAR, UV y por técnicas de tamizaje fitoquímico⁶. Los compuestos principales fueron aislados del extracto seco mediante fraccionamiento líquido/líquido, cromatografía líquida semipreparativa y la identificación se realizó por el análisis de los espectros RMN. La actividad antitumoral fue evaluada en modelos in vitro e in vivo de cáncer colorrectal con células CT26W.T.

Resultados / Discusión / Conclusión

Los parámetros de calidad evaluados se encontraban dentro de los valores generales establecidos para drogas vegetales. Los perfiles CCD, UV, HPLC y los resultados del tamizaje fitoquímico mostraron la presencia de fenoles, punicalaginas y ácido elágico como compuestos mayoritarios. No se evidenciaron diferencias

significativas en el contenido de polifenoles totales, en ambos extractos, demostrándose que el proceso de secado por aspersión no afecta el contenido de estos metabolitos. Tres compuestos fueron aislados del extracto seco siendo la Punicalagenina, el compuesto mayoritario. El extracto demostró ser citotóxico para las células CT26W.T y presentó actividad antimetastásica en el modelo de metástasis pulmonar inducida. El extracto seco de *Punica granatum* L se considera un candidato potencial para el desarrollo de nuevos productos antitumorales a partir de plantas medicinales.

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Efecto antimicrobiano *in vitro* de los extractos hidroetanólicos de hojas de *Prosopis pallida* (Algarrobo), *Ruta graveolens* (ruda) y *Plantago major* (Llantén) sobre microorganismos de importancia Estomatológica

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Introducción

La caries dental y la enfermedad periodontal son enfermedades orales con alta prevalencia a nivel mundial. Sus agentes causales son principalmente microorganismos o sus productos. El control de estos patógenos es principalmente químico a través de antibióticos o colutorios. El uso indiscriminado de antimicrobianos y otros factores ha propiciado la manifestación de resistencia en los microorganismos, convirtiéndolos en un problema de salud pública mundial. Las plantas medicinales han sido siempre una potencial fuente de nuevos fármacos en las sociedades. En la región norte del Perú se han descrito más de 50 plantas medicinales, cuyas propiedades antimicrobianas sobre microorganismos orales aún no han sido estudiadas completamente.

Método

El objetivo de la presente investigación fue evaluar el potencial antimicrobiano *in vitro* del extracto hidroetanólico de hojas de *Prosopis pallida* (Algarrobo), *Ruta graveolens* (ruda) y *Plantago major* (Llantén) sobre *Candida albicans* ATCC 10231, *Streptococcus mutans* ATCC 35668 y *Porphyromonas gingivalis* ATCC 33277. Los extractos totales se obtuvieron por maceración hidroetanólica. Los inóculos bacterianos se estandarizaron espectrofotométricamente a $1,5 \times 10^8$ UFC/mL. Se evaluaron 10 concentraciones en $\mu\text{g/mL}$ de los extractos de cada planta estudiada, más los controles clorhexidina al 0,12% (antibacteriano) y Nistatina 100.000 UI (antifúngico). El potencial antimicrobiano se evaluó mediante el método de discodifusión. La concentración mínima inhibitoria (CMI) y la concentración Mínima bactericida (CMB) y fungicida (CMF) mediante el método de microdilución.

Resultados / Discusión / Conclusión

Se reportó efecto antimicrobiano en las diferentes concentraciones de los extractos de las tres plantas evaluadas sobre los tres microorganismos enfrentados. La CMI de los extractos hidroetanólicos de *P. pallida*, *P. major* y *R. graveolens* sobre *Candida albicans* ATCC 10231 fue 700 $\mu\text{g/ml}$ y la CMF fue 800 $\mu\text{g/ml}$. En el caso de *S. mutans* ATCC 35668 la CMI y la CMB de *P. pallida* fue 100 $\mu\text{g/mL}$, la de *P. major* fue de 900



$\mu\text{g/mL}$ y $1000 \mu\text{g/mL}$ y la de *R. graveolens* fue de $600 \mu\text{g/mL}$ y $700 \mu\text{g/mL}$. La CMI y CMB de *P. pallida* sobre *P. gingivalis* ATCC 33277 fue $100 \mu\text{g/mL}$ y $200 \mu\text{g/mL}$, la de *P. major* fue $200 \mu\text{g/mL}$ y $300 \mu\text{g/mL}$ y la de *R. graveolens* fue $100 \mu\text{g/mL}$ respectivamente. Se concluye que, las diferentes concentraciones de los extractos probados tienen efecto antimicrobiano *in vitro* de tipo bactericida sobre *S. mutans* ATCC 25175 y *P. gingivalis* ATCC 33277 y de tipo fungicida sobre *C. albicans* ATCC 10231. Dicho efecto fue estadísticamente significativo respecto a los controles.

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***Cladanthus arabicus* and *Bubonium imbricatum* from Morocco: chemical properties and antibacterial activity of essential oil and extracts**

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Introduction

The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents. Recently, medicinal plants and their derivatives have become very important in therapeutics because they encounter minimal challenges of the emergence of resistance. In this direction the antimicrobial activity of the endemic *Bubonium imbricatum* plant and medicinal *Cladanthus arabicus* plant essential oils and extracts against multidrug resistant *Enterobacteriaceae* strains was demonstrated, together with polyphenols and major and trace elements of their extracts.

Method

UV spectrophotometry, ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) and inductively coupled plasma mass spectrometry (ICP-MS) were used to evaluate total polyphenol content, quali-quantitative profile of single polyphenols and inorganic elements of the extract. Standard methods, such as disc diffusion assay, Minimal Inhibitory Concentration (MIC) and checkerboard test were used to investigate the antibacterial activity.

Results / Discussion / Conclusion

The aerial parts of *B. imbricatum* and *C. arabicus* were thoroughly analyzed for total polyphenol contents, single polyphenols, major and trace elements, including heavy metals, and antibacterial activity.

Twelve polyphenols were identified in both plants and these were more concentrated in *B. imbricatum* than *C. arabicus* extracts. High levels of minerals, essential trace elements and tolerable levels of heavy metals

(Cd, As and Pb) were found. Additionally, in vitro antibacterial activity of *B. imbricatum* than *C. arabicus* extracts against a collection of MDR Enterobacteriaceae, particularly versus *E. coli* S33/16 (MIC, 125 $\mu\text{g ml}^{-1}$), that may be explained by the presence of certain polyphenols, was reported.

Also *B. imbricatum* and *C. arabicus* EOs showed a potent activity against the tested Enterobacteriaceae isolates, with inhibition zones values in the range of 8.05 ± 0.1 and 13.1 ± 0.11 mm and MIC values between 200 $\mu\text{g ml}^{-1}$ to 800 $\mu\text{g ml}^{-1}$ for *C. arabicus* and from 400 $\mu\text{g ml}^{-1}$ to 1600 $\mu\text{g ml}^{-1}$ for *B. imbricatum* respectively.

This study allowed concluding that both EOs showed also active effects combined with conventional antibiotics,

demonstrated by the Fractional Inhibitory Concentration Index

Obtained results should encourage further in vivo studies for the development of phytoterapeutic products.

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Research initiatives at Takiwasi Center on drug addiction, mental health and traditional Amazonian medicine

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Introduction

Takiwasi Center is a not for profit private institution located in Tarapoto, Peru, working for more than 25 years for the treatment of people confronting problems of drug addiction and mental health integrating the Traditional Amazonian Medicine (TAM) with modern approaches including psychotherapy [1]. Takiwasi acts as a therapeutic community authorized by the regional health authority and as a research center recognized by the National Council for Science, Technology and Technological Innovation. In the present work we present the main research project results with particular emphasis on those related with ethnomedicine and phytotherapy.

Method

Internal data system management [2] and other informatics tools available at Takiwasi were consulted in order to acquire qualitative and quantitative information on the main research activities performed overtime on drug addiction, mental health, and herbal medicines by internal personnel and visiting scholars. Major publications including peer reviewed articles published by the center's affiliated were also recorded and discussed.

Results / Discussion / Conclusion

Despite the relatively recent creation of this center and its limitations in terms of personnel and resources dedicated to research programs, the overall number of activities, projects, and outputs clearly highlight the international dimension and the multidisciplinary approach of the research performed at Takiwasi. Typical initiatives well fit into the field of ethnomedicine and phytotherapy, where the use of the psychoactive brew known as Ayahuasca, a complex formula composed by *Banisteriopsis caapi* (Spruce ex Griseb.) Morton ([Malpighiaceae](#)) most often complemented by *Psychotria viridis* Ruiz & Pav. (Rubiaceae), represent only one of the many herbal TAMs used within the health service programs of the center. Such therapeutic practices have been analysed and discussed by many researchers having different backgrounds including psychology, biochemistry, pharmacy, anthropology, music therapy between others. This data indicates the necessity for a



multiple perspectives approach to describe this complex therapeutic protocol that is based on the integration of modern medical tools with the ancient knowledge of traditional Amazonian healers. However, many scientific questions still wait for answers and additional efforts are needed for further advances especially in the area of drug addiction, mental health and herbal TAMs.

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Evaluation of cyanotoxins in edible cyanobacterial colonies from the andes wetlands in South America

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Introduction

Edible colonies of the cyanobacterium *Nostoc* sp., grown at Andean wetlands, are sun-dried and sold as Llayta. Llayta is today used as an ingredient for human consumption in southern Peru and northern Chile; a feeding practice that can be traced back to pre-Columbian times (Rivera et al., 2018). The cyanobacterial biomass is rich in essential amino acids for humans (60% of total amino acids) and polyunsaturated fatty acids (32% of total fatty acids) (Galetovic et al., 2017). Since some members of the *Nostoc* genus are cyanotoxins producers (e.g., microcystin LR) (Sivonen et al., 1990), this work provides analytical and genomic evidence showing that Llayta is a cyanotoxin-free biomass.

Method

Llayta was bought (several plastic bags containing about 100 g each) at the food market in Arica, Chile. Samples (10 mg) of the dry biomass were extracted with 50% methanol (Merck, HPLC grade) and subjected to HPLC-MS analyses. DNA from the Llayta biomass was extracted using the kit Ultra Clean Microbial DNA (MoBio) to learn, by PCR and genomic analyses, if key genes (e.g., *mcyA* and *mcyE*) involved in the biosynthesis for cyanotoxin Microcystine-LR (MC-LR) were present in the Llayta genome.

Results/ Discussion/ Conclusion

HPLC chromatograms from standard MC-LR showed one peak with a retention time (R_T) of 7.2 min, absorbance maximum at 238 nm, and $[M+H]^+$ ion mass fragments of 995.37, 977.36, 866 and 599 m/z, as expected for microcystin LR (Lawton et al., 1994; Ramadan et al., 2000). In contrast, the HPLC chromatogram obtained from methanolic Llayta extracts did not show the signal at R_T 7.2 min. In addition, the MS analysis of the Llayta HPLC signal region at R_T 6-7 min provided a different ion mass fragment pattern. However, the Llayta extract showed a strong signal at R_T 3 min which deserves further investigation to determine its nature. Genomic DNA was extracted from Llayta to learn if key genes, *mcyA* and *mcyE*, involved in the biosynthesis of cyanotoxin MC-LR were present in the Llayta DNA. Only gene *mcyE* was

amplified by PCR using the purified DNA from Llayta (positive control: *Microcystis aeruginosa* LEGE 91339). After recovering, sequencing and blasting (BLASTn) against sequences at the NCBI data bank, the Llayta amplicon showed a 96% identity with *mcyE* gene sequence registered for the cyanotoxic *Nostoc* sp. strain 152 (Sivonen et al., 1990). These results can be interpreted as (a) the *mcyE* gene is present in the Llayta DNA but its expression is controlled by one or more still undetermined environmental factors or (b) the *mcyE* gene amplicon sequence observed in the Llayta DNA would share deoxynucleotide sequences with genes coding for enzymes, also dependent of the non-ribosomal biosynthesis pathway, that synthesize products other than MC-LR. We consider that these results are evidence for the absence of microcystin LR in the Llayta extracts but caution and further studies are necessary if the biotechnological use of *Nostoc* sp. strain Llayta is considered. BMAA, a non-proteinaceous amino acid found in several members of cyanobacteria genera, including *Nostoc* genus, and the probable agent of neurological disorders (Baptista et al., 2011; Monteiro et al., 2017). Our work is presently addressing this issue in Llayta.

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Estudio químico y efecto antilitiásico de *Caesalpinia bahamensis* Lam

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Introducción

Caesalpinia bahamensis es una planta medicina empleada tradicionalmente en Cuba para el tratamiento de enfermedades renales y hepáticas, diabetes mellitus y úlceras crónicas¹. Sin embargo, a pesar del amplio uso que tiene en la población los estudios científicos sobre la especie son escasos. En este estudio se determinaron los parámetros de calidad del tallo de *C. bahamensis*, se realizó la caracterización de los extractos acuoso e hidroalcohólico, se aislaron e identificaron los compuestos mayoritarios del extracto hidroalcohólico y se evaluó su efecto antilitiásico.

Método

Los parámetros de calidad de la droga fueron evaluados siguiendo los procedimientos establecidos por la OMS (1992)². Los extractos fueron caracterizados mediante sus perfiles CCD, CLAR, UV y por técnicas de tamizaje fitoquímico³. Se cuantificaron los fenoles totales mediante el método de Follin-Ciocaoulteu y los flavonoides mediante el método del tricloruro de aluminio⁴. Los compuestos principales fueron aislados del extracto hidroalcohólico mediante fraccionamiento líquido/líquido, cromatografía flash y cromatografía líquida semipreparativa y la identificación se realizó por el análisis de los espectros RMN. El efecto antilitiásico fue evaluado en ratas Wistar hembras empleando el método de inducción del cálculo con etilenglicol y cloruro de amonio⁵.

Resultados / Discusión / Conclusión

Los parámetros de calidad evaluados se encontraban dentro de los valores generales establecidos para drogas vegetales. Los perfiles CCD, UV, HPLC y los resultados del tamizaje fitoquímico mostraron la presencia de flavonoides y fenoles como compuestos mayoritarios. La cuantificación de fenoles fue superior para el extracto acuoso y la de los flavonoides para el extracto hidroalcohólico. Siete compuestos fueron aislados del



extracto hidroalcohólico siendo la Protosapanina B el compuesto mayoritario. Ambos extractos mostraron actividad antilitiásica en el modelo evaluado, lo cual convierte a la planta en un potencial candidato para estudios posteriores que conlleven a su aplicación en la clínica. Todos los resultados presentados se reportan por primera vez para esta especie.

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Phytochemical and chemical characterization of metabolites by gas chromatography / mass spectrometry of the toasted cocoa shell

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Introduction

The cocoa husk is of a crunchy nature and brown in color, it represents approximately 12% of the mass of the roasted cocoa beans (Kalvatchev et al., 1998), other authors describe it as the testa is obtained by husking the cocoa bean, It has a brown color and a characteristic smell of cocoa (Bustamante, 2014). A study conducted by Lecumberri et al. (2007) on the chemical characterization of by-products of the cocoa industry of a locality in Ecuador that included the husk, reports that it has a high content of dietary fiber, around 50% m / m, and an important antioxidant activity. Therefore, this research had as a general objective to characterize phytochemical and chemical roasted cocoa husk by gas chromatography / mass spectrometry.

Method

For this, the total phenol and flavonoid content was calculated by the Folin-Ciocalteu methods (Singleton et al., (1965) and AlCl₃ Chang et al., (2002) and Pourmorad et al., (2006). The ethanolic extract 90% and aqueous were analyzed using a gas chromatograph coupled to a Shimadzu QP2010 Ultra mass spectrometer after derivatizing it with BSTFA. The pulverized sample was evaluated through a phytochemical screening to obtain information about the chemical components present in the sample.

Results / Discussion / Conclusion

The content of total phenols and flavonoids in the cocoa husk was 4,12 mg of gallic acid / mL and 1,43 mg of quercetin / mL. The GC-MS analysis revealed the presence of 74 from the husk. According to the database of the NIST21 and NIST107 libraries, some of the chemical compounds found were Caffeine, Theobromine, Catechin, Uridine, Stigmasterol, Cholesterol and Androstane-11,17-dione, 3 - [(trimethylsilyl) oxy] - , 17- [O- (phenyl methyl) oxime]. Phytochemical screening allowed to suggest the possible presence of oils and /

or fats, alkaloids, triterpenes and / or steroids, catechins, reducing sugars, saponins, phenolic compounds, amino acids or amines, quinones, anthocyanidins, flavonoids and bitter or astringent principles. The information obtained from these studies can be used as chemical markers in the standardization and identification of the cocoa husk that can be used as a functional ingredient in food formulation.

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Phytochemical study and evaluation of the antioxidant capacity of flowers of *Senna spectabilis* in Colombia

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Introduction

Senna spectabilis (Fabaceae) possesses a great medicinal value, reason for which has been used to treat headaches, inflammation, flu and injuries; It is traditionally known as “yellow flower” and is distributed mainly in the departments of Cundinamarca, Tolima and Antioquia in Colombia. For this species (mainly in bark, leaves and roots) have been determined numerous chemical substances as alkaloids, flavonoids and polyphenols with bioactivity such as antifungal, antibacterial, antioxidant, anticonvulsant, and sedative (Kamo, Maehara, Sato, & Hirota, 2003; C. Viegas et al., 2004; Jäger, Trojan, Kopp, Laszczyk, & Scheffler, 2009; Silva Oliveira, de Vasconcelos, & Braz-Filho, 2010). The purpose of this work was to make a contribution to the chemical and biological knowledge of the flowers of *Senna spectabilis* through the chemical study of the fixed and volatile metabolites, and the evaluation of their antioxidant capacity.

Method

The collection of the flowers of *Senna spectabilis* was made in the municipality of Guaduas-Cundinamarca (5 ° 05'16,4 "N 74 ° 36'54,3" W) and they were identified in the Colombian National Herbarium under the collection number COL 600165. From the ethanolic extract obtained by cold maceration, the fractions of heptane, dichloromethane, ethyl acetate, alacaloidal and a hydroalcoholic residue were obtained. Each of these was subjected to continuous separations through column chromatography, thin layer and preparative thin layer, allowing tentative identification by gas chromatography coupled to mass spectrometry (GC-MS) of three mixtures and two Compounds, reported by first time for this species in our country. The essential oil was obtained by steam distillation from fresh flowers, its determination was made by GC-MS, comparison of retention indexes, mass spectra and data reported in the literature. The antioxidant capacity was determined by the DPPH method to the extract and fractions obtained. The statistical analysis for the quantification of the antioxidant capacity (% inhibition and IC50) was performed using the software statgraphics centurion XVI, where correlation coefficients, analysis of variance and correlation of the variables were established for each of the evaluated treatments.

Findings / discussion / Conclusion

The separation and purification of the fractions of the flowers of *Senna spectabilis* led to the isolation of two compounds identified as caffeine and methyl cinnamate and three mixtures, the first consisting of two esters (ethyl 9-oxo-nonanoate and ethyl palmitate) and a ketone (pentadecan-2-one); the second mixture consisting of ethyl palmitate and an anthraquinone (1,8-dihydroxy-3-methyl-anthraquinone) and the third constituted by linalool and methyl cinnamate. The essential oil was obtained with a yield lower than 0.1%, where the presence of 21 compounds was determined (53.27%) being ethyl palmitate the majority compound (29.94%). The determination of the antioxidant capacity allows to establish that only the total extract and the fractions of dichloromethane and ethyl acetate have a percentage of inhibition higher than 30%.

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Accumulation of isoflavonoid phytoalexins in colombian common bean (*Phaseolus vulgaris*) cultivars in response to treatment with 1-oxo- indanoyl- (*L*) - isoleucine methyl ester

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Introduction

Plants have developed highly effective and complex chemical systems to defend themselves against microbial and herbivores pathogens. These defensive responses are associated with the accumulation of secondary metabolites (*phytoalexins*), which are responsible for reducing damage in plants and increasing resistance to pests, diseases or environmental stress. The recognition of plant cells to attacks by pathogens or herbivores depends on a diverse group of structural compounds (extrinsic or intrinsic) that act as signaling molecules called **elicitors**. An example for these class of substances is the 6-ethyl indanoyl isoleucine conjugate (**coronalone**), which is known as a synthetic elicitor that has been shown to be effective in a series of biological responses associated with plant development and defense. The synthetic elicitors have proved to be as a promising alternative to conventional biocidal pesticides. For the above, we carried out the synthesis of a coronalone analog (**1-oxo-indanoyl-(L)-isoleucine methyl ester**) and proved its effectiveness as elicitor by the accumulation of isoflavonoide phytoalexins in Colombian bean varieties. This research was carried out in a vegetable species of great nutritional and commercial importance for the world and specially for Colombia. It is expected to contribute information to breeding programs in beans, based on the production of phytoalexins.

Method

The 1-oxo-indanoyl-(L)-isoleucine methyl ester was synthesized from 2-carboxy-hydroxycinnamic acid in three steps: catalytic hydrogenation, friedel-crafts acylation and aminoacid esterified (L)-isoleucine coupling. Each reaction product was purified by chromatographic techniques (TLC, CC and HPLC) and identified by spectroscopic and spectrometric methods (¹H and ¹³C NMR , UV andMS). The evaluation on the accumulation of phytoalexins was carried out by HPLC, which were previously isolated, purified and identified by chromatographic and spectroscopic methods. The analyzes involved the induction of phytoalexins in different bean varieties (ICA Quimbaya, Cargamanto rojo, Cargamanto blanco), different

tissues of the plant (cotyledons and hypocotyl/root), evaluation of course of time and antifungal activity. The antifungal activity was determined by inhibition of mycelial growth against *F.oxysporum*.

Results

The accumulation of phytoalexins depends on the type of bean (the resistant variety to fungal diseases produced higher contents of isoflavonoids than the susceptible variety); the tissue of the plant (cotyledons accumulated a greater content of phytoalexin than the hypocotyl or root), and the response in the time (maximum accumulation of isoflavones during the 24 and 48 hours post induction, and maximum accumulation of pterocarpan between 72 and 96 h post induction). The compound shown a radial growth inhibition of 52%, suggesting that has a fungistatic and non-fungicidal effect.

Conclusion

The exogenous application of the elicitor **1-oxo-indanoyl-(L)-isoleucine methyl ester** on bean plants, has been suggested as a good alternative to increase the contents of isoflavonoids phytoalexins, and subsequently a good candidate for the design of new phytoprotectants in crops.

Chemical biological study of *Premna resinosa* (hochst.) schauer surface extract

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Introduction

Premna resinosa (Hochst.) Schauer belongs to the Lamiaceae family. This genus includes about 200 species mainly distributed throughout tropical and subtropical Asia, Africa, Australia, and the Pacific Islands (Dianita et al., 2017; Rekha et al., 2015). Phytochemical studies performed in some *Premna* species revealed existence of diterpenoids, triterpenoids, iridoid glycosides, flavonoids, lignans and xanthones (Dianita et al., 2017; Rekha et al., 2015). Some *Premna* species are considered as natural resources for antimicrobial, antioxidant, antipyretic, hypoglycaemic, cardiogenic, and diuretic agents (Hymavathi et al., 2009). The essential oil obtained from *Premna* species displayed antibacterial properties (Rekha et al., 2015). *P. resinosa*, a shrub or small bushy tree, with whitish stems and coriaceous leaves with a pleasant smell, is used in Indian traditional medicine, as laxative agent and to treat bronchitis. The extracts of *P. resinosa* and their flavonoid constituents showed cytotoxic, anti-tubercular, and antimicrobial activities (Rekha et al., 2015; Albadawi et al., 2017).

Based on previous reports about *Premna* genus pharmacological activities and in continuation of our ongoing research project focused on bioactive compounds from interesting medicinal plants, *P. resinosa* was selected in this study.

In this investigation we report the isolation and characterization of labdane diterpenes of the surface extract of *P. resinosa*. and the evaluation of their antiproliferative effects.

Method

The dichloromethane extract of the surface mixture, obtained from the fresh aerial parts of *P. resinosa*, was separated by different chromatographic techniques such as Silica gel, MPLC, and HPLC. The structures of isolated compounds were elucidated by 1D and 2D NMR and MS Spectroscopy. The antiproliferative activity of the isolates was investigated on Jurkat and HeLa cell lines through MTT assay.

Results / Discussion / Conclusion

The phytochemical investigation of surface extract afforded 5 new labdane-type diterpenes and along with three methoxylated flavones and two diterpenes already described in the literature. The antiproliferative activity of the isolates was investigated on Jurkat and HeLa cell lines. The most active diterpene (**1**) at 48 h showed activity in both cell lines (IC_{50} of $13 \pm 0.7 \mu\text{M}$ and $16 \pm 0.9 \mu\text{M}$, respectively).

Our results indicate that *P. resinosa* surface extract is a source for bioactive labdane diterpenes.

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Chemical characterization of ecuadorian propolis by HPLC-PDA-MS/ESI

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Introduction

Propolis is produced by bees from plant sources, mainly. Its chemical composition is variable and depends on the vegetation that the insect visits to collect resins, exudates or parts of plants. Propolis from some geographical areas has been very well characterized: some samples of temperate zones are characterized by the presence of flavonoids, organic acids and their derivatives. Isoflavonoids are considered as major components of some samples from tropical areas of America and Africa and some prenylated phloroglucinols have been reported in tropical countries, among others (Bankova et al., 2019).

Chemical composition of Ecuadorian propolis has been little studied and the biodiversity of Ecuador assumes the existence of a great variety from a chemical point of view (Cuesta-Rubio et al., 2017). For these reasons, a comparative study of 16 samples of propolis by HPLC-PDA-MS/ ESI is carried out in order to identify similarities and differences between these samples and the main type of propolis known internationally.

Methods

Propolis samples were extracted with methanol by maceration (3 x 4 h). The extracts were concentrated to dryness under reduced (35 °C) and 10 mg of the solid residue was dissolved in HPLC grade methanol (1 ml). Solutions were filtered employing a 0.2 µm filter and analyzed without further treatment using an Ultimate 3000 liquid chromatography system (Thermo scientific) consisting of a quaternary pump, an auto sampler, a column compartment, PDA detector and LTQ XL mass spectrometer equipped with an ESI source and a linear ion trap analyzer.

Separations are developed on a Thermo Scientific 2,6 µm Accucore RP-MS column (100 x 2,1). The elution solvents used were water and acetonitrile. The flow rate was 0.4 mL/min and PDA data were recorded with a 200-600 nm range with four preferential channels as the detection wavelength, 214, 250, 280 and 330 nm. The mass spectrometer operated in both negative and positive ion mode in the 100-1000 Da range. Experiments were performed in dependent scan mode to obtain the MS/MS spectra.

Result / Discussion / Conclusion

The chromatographic profiles and mass spectra data allowed to identify at least 5 different groups of Ecuadorian propolis respect to its chemical composition. The compounds that originated the most intense chromatographic signals showed pseudomolecular ions below 700 Da, with negative ion mode exhibiting the best results. More than 40 components were identified in 16 samples of propolis. Some samples showed a behavior similar to temperate propolis due to the presence of chrysin, apigenin, pinobanksin esters and organic acids. Likewise, sample rich in diterpenic acids and triterpenic acids were also detected. Some propolis samples appear to contain novel components that merit their isolation and unequivocal structural characterization. Chemical components suggested that bees visit dissimilar plant sources among which *Populus* and *Mangifera* species seem to be included.

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Evaluation of broad spectrum photoprotective activity of *Pentacalia pulchella* (Asteraceae) extracts, and potential use in functional cosmetics

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Introduction

Ultraviolet radiation (UVR) has harmful effects in humans, and these effects depend on the intensity of radiation, exposure time and UVR range (UVA, UVB). Additionally, the intensity of UVB radiation increases with the decrease of the ozone layer. According to data from the world health organization every year in the world there are 2-3 million new cases of non-melanoma and 132,000 of melanoma cancer (de Gruijl, 1999; Halliday et al., 2004). On the other hand, the biological diversity found in the high mountain ecosystems of the tropics is unique; because most of their plant species have developed complex adaptations to survive in the extreme climatic conditions (high levels of UVR and low temperatures) of these ecosystem (Agati et al., 2013). In a preliminary study in some plants of this ecosystem in Colombia, we found that one of the most promising species was *P. pulchella*, an endemic plant of Colombia, which grows between 2500-3000 m.a.s.l. Consequently, in this work focused on the search for new natural sources of photoprotection, for which the photoprotective, antioxidant and chemical composition of methanolic and acetone extracts from the leaves of *P. pulchella* was evaluated.

Method

All assays were performed according to a complete factorial design (2³) and each assay was done in triplicate. The analysis of the main components presents in each one of the extracts was done in an HPLC-DAD and UPLC-ESI-IT-MSⁿ. *In vitro* photoprotection was evaluated according to the following parameters: UVA-UVB absorption spectrum, SPF, UVAPF, λ_c , UVA/UVB ratio. The antioxidant activity was determined using the DPPH radical assay and the inhibition of lipid peroxidation in methyl linoleate. In addition, the Total Phenols Content (TPC) was evaluated by the Folin-Ciocalteu method (Mejía-Giraldo et al., 2016).

Results / Discussion / Conclusion

According to the analysis of the variance of three factors (factorial design 2^3), methanol and the addition of acid increased the percentage of overall yield of the extracts, and the addition of activated carbon decreased it. The extracts in general showed good absorption coefficients in UVA-UVB, a high TPC, with antioxidant activity comparable to that obtained with butylhydroxytoluene (BHT). Finally, the formulations in hydrogel based on ethanol-propylene glycol-water extract, presented sensory characteristics suitable for topical use and good protection values in the UVA-UVB range. Regarding to the evaluation of the broad spectrum protection of the extracts of *P. pulchella*, a good protection was found in the UVA range, according to the parameters of COLIPA and FDA. All the λ_c were higher than 370 nm, and the FPUVA values were greater than one third of the FPS values found, which allows to declare UVB-UVA broad spectrum protection in the formulations. The results obtained suggest that the extract of *P. pulchella* has a high potential as a source of new natural sunscreens.

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Efficacy of dietary supplements Alzer® and Diamel in the prevention of severe diabetic macular edema. Phase II clinical trial

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Introduction: Macular edema is the main cause of low vision in diabetic patients. There is no medical treatment that has proven effective in this condition, laser photocoagulation remains the treatment of choice, associated with the use of steroids and anti-angiogenesis, but they are not exempt from possible eye complications. The nutritional supplement Alzer whose main active ingredient is Ginkgo biloba, a powerful antioxidant that acts on vascular factors and oxidative damage that are two mechanisms involved in the pathogenesis of diabetic macular edema, used in other non-diabetic macular conditions, could represent together with the Diamel nutritional supplement, which has shown efficacy in glycemic control, an alternative treatment in mild and moderate macular edema, reducing the thickness of the macular retina and preventing progression to other more advanced and difficult to treat clinical forms. **Objective:** To identify the effect of the Alzer + Diamel (A-D) in the reduction of the retinal macular thickness in patients with mild and moderate diabetic macular edema. **Material and method:** A phase II, double-blind, placebo-controlled clinical trial was conducted in 64 patients with non-severe diabetic macular edema in the course of a non-proliferative diabetic retinopathy, the treatment will be randomly assigned to two groups, one you will receive the Alzer + Diamel and the other group will receive placebo from Alzer + Placebo from Diamel. All the patients underwent an initial clinical, biochemical and ophthalmological evaluation, at the beginning and the year of treatment. **Results:** There was clinical improvement in macular thickness at the conclusion of the study although these changes were not statistically significant. There was no decrease in visual acuity. Adverse events were few and mild. **Conclusions:** The cases of group A-D did not evolve to a severe EMD. The success obtained clinically but not statistically in group A-D fulfills the hypothesis of the protocol on the efficacy of the research product. The positive results in this small sample make us propose studies on a larger scale (Phase III).

Effectiveness of the Diamel associated with insulin in the treatment of diabetic foot ulcer

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More than 25% of diabetic patients may develop one ulcer on foot and for this reason may suffer one amputation. Hyperglycemic state and oxidative stress contributes to develop foot lesion . Diamel one nutraceutical food may increase insulin sensibility in patients with and without Diabetes Mellitus (DM).

Objective: to identify the effectiveness of the Diamel associated with insulin in the treatment of diabetic foot ulcer.

Subjects and methods: clinical trial (phase II) randomized double-blind. Subjects: n = 100 both sexes, with DM, Ulcer of the foot grade I and II of Wagener and absence of disabling disease severity. They received during a year: a) Diamel 6 tablets/day (n=50 patients study group) and b) placebo received similar tablet in appearance without the Diamel components (n=50) tablets/day. In all patients was determined : i) control glycemic (HbA1c) prior to the start of treatment and every third month, ii) local healing of the lesion was performed ,iii) began treatment with insulin action intermediate dose needed to achieve glycemic control. Primary objective demonstrate a cure $\geq 25\%$ (healing of the injury and time occurred) between both groups, as well as injury recurrence $< 5\%$. Secondary objective to identify undesirable effects (frequency and types). Statistical analysis: statisticians of central tendency and logistic regression.

Results: We lost 20 patients were during study period 6 by causes medical and 14 by missing . Of the 80 patients who completed study the first month differences between groups regarding the frequency of healing there was 30 vs 24% between study vs placebo, the 2nd month 88 vs 30% (p = 032), the third month healing there was 77% of the group control vs 52% group placebo (25% difference intergroups p = 0, 044). No adverse effects were reported. It showed not differences between clinical variables (age, sex, type of DM, smoking), although HbA1c levels were lower in patients from the study group.

Conclusions: The Diamel product in patients with diabetic foot ulcers treated with insulin, showed superiority in the healing time, in the non-emergence of new lesions, not adverse effects and better glucemic control.

Key words: diabetic ulcer foot, Diamel, metabolic control , insulin

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Effects of *Moringa oleifera* on bone mass

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Osteoporosis is the most frequent bone metabolic disease, for which we have a therapeutic arsenal that presents multiple adverse effects and a high cost, for which it is necessary to have other therapeutic alternatives, a bibliographic review was carried out in order to look for alternatives natural products that improve bone mass, minimizing costs and adverse effects. We conducted a search in pubmed, free medical journal, Ebsco and Hinari, using as key words: bone mass or osteoporosis and natural and traditional medicine or moringa. Four papers were found in which the possible beneficial effects of the different components of *Moringa oleifera* (MO) on bone mass were explored. MO has anti-inflammatory, antioxidant, antimicrobial, antihyperlipidemic, antifertility and anticancer activities. An in vivo study demonstrated the osteoprotective potential of the plant. Another study attempted to explore the osteoblastogenic potential of different parts of the plant in the osteoblast cell line SaOS2. The methanolic extracts of the MO components showed a positive effect, and of the three components, it was discovered that the flowers and fruits had significant properties of osteoblastic stimulation. It was found that the flower extract increased the number of osteoblastic cells; while the fruit extract induced bone formation, increased hydroxyproline content and bone mineral formation. In a study conducted in postmenopausal women (60 to 70 years) it was concluded that there is no evidence in the short term supplementation of MO in bone mineral density (BMD). Another investigation in diabetic osteoporotic rats provided the first information about the probable effect of MO and its different components, this showed that the consumption of this plant can have a beneficial effect in the improvement of these two diseases. The results obtained provide evidence that MO fruit extracts contribute significantly to the prevention of bone loss in diabetic rats with osteoporosis. Finally, a study carried out in ovariectomized rats with Etanolic Extact of MO found that it could restore the reduced level of calcium, correct the high rate of bone turnover, reduce the elevated level of alkaline phosphatase, confirming its beneficial effects and its potential to prevent osteoporosis naturally through herbal resources. Therefore, we conclude that MO could be a future therapeutic alternative to improve bone mass.

Histopathological study of the effect of vimang cream in the deep second degree burns cicatrization process

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ABSTRACT

Introduction: The burn is a traumatic wound with local and systemic damage, as well as oxidative changes. Vimang possesses antioxidant, anti-inflammatory and antibacterial activity. In addition, their compounds intervene in the improvement of the epidermal barrier and reduce the contraction of wounds, contributing in a beneficial way in the cicatrization process. To evaluate histopathologically the effect of Vimang cream in the cicatrization process of deep second degree burns was de objective.

Materials and Methods: Prospective longitudinal study of individuals with deep second degree burns was carried. They were divided into two groups, both treated with conventional treatment and another, with Vimang cream was added. A biopsy was performed on day 14. Healing time and the histological signs related to the four phases of the healing process (Hemostasis, Inflammatory, Proliferative, and Maturation) constituted the variables of the study. Descriptive measures were determined and the groups were compared using the proportions comparison test. The 95% confidence intervals were determined.

Results and Discussion: All patients in the group with Vimang cream shortened the healing time to 14 days and only 5.26% of the conventional group. The group with Vimang presented advanced proliferative phase, presence of collagen fibers, fibroblasts and lymphocyte proliferation. Complete regeneration of epidermis, visible basal lamina through PAS staining. The conventional treatment group presented initial proliferative fibroblastic stage with acute elements at the dermal level. The epithelization phase was completed in 50% of the sample. **Conclusion:** Vimang cream was beneficial in the proliferation of fibroblasts, lymphocytes and decreased granulation tissue during the healing process of deep second degree burns.

Intervention with shark cartilage, for 1 year. An effective antioxidant in metabolic syndrome

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Summary:

Introduction: The evolution of the approaches for the metabolic syndrome from the original definition of the World Organization of the Health in 1998 reflective the growth of the clinical evidence and analysis on the part of diverse conferences of consents and professional organizations. The main characteristics of the metabolic syndrome include central obesity, hipertrigliceridemia, low concentrations of cholesterol of lipoproteína of high density (HDL, high-density lipoprotein), hiperglucemia and hypertension, are a continuous flow of new results of investigations that you/they evidence that the speceies reactive oxygen makes a significant contribution to the progression of the diabetes and their complications, recent studies they reflect the anti-rust properties of the shark cartilage fundamentally in the lípidos protection, due to their chemical composition. **Materials and methods:** 30 patient payees of the metabolic syndrome were selected and they were determined the variable hemoquímicas and of estrésoxidativo. **Results:** Significant decreases of the concentrations of Maloniladehído were obtained, total Organoperóxidos and advanced products of the oxidation of proteins. **Conclusions:** A 1 year-old therapy conditions an improvement in the markers of estrés oxidative

Keywords: Metabolic syndrome, stress oxidative, cartilage shark.

PC6 acupoint modifies autonomic standing response in diabetic patients

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Introduction

Diabetes and other chronic diseases elicited progressive autonomic dysfunction. Orthostatic hypotension (OH) is one of the more striking dysfunctions of AD. The autonomic response to the standing challenge is a critical adaptative response in humans. Many people especially those with autonomic dysfunctions can experiment severe symptoms to the stand position. Pericardium 6 (PC6) acupoint has shown to modify autonomic nervous responses. The objective of this study was to compare the changes in the 30:15 ratio to the active standing, before and after the application of acupuncture in the PC6 acupoint.

Method

The 30:15 ratio of healthy subjects (n=15, 44.6 years old) versus type 2 diabetic subjects (n=15, 48.3 years old) were compared. Subjects were made to lie quietly on a couch while the heart rate is continuously monitored on an electrocardiograph. The subject is then asked to stand unaided and the point at the starting to stand is marked on the electrocardiograph. The shortest RR interval at or around the 15th beat is measured, while the longest RR interval at around the 30th beat after standing is measured. Both groups underwent two consecutive tests of active standing test (AST) moving from lying supine to standing position, before and after manual acupuncture treatment. The statistical analysis of this function test was done by using the unpaired t-test to compare the autonomic nervous system activity of the diabetic patients and the normal healthy controls. A p value of <0.05 was taken as significant.

Results / Discussion / Conclusion

Acupuncture in PC6 elicited a significant increase ($p = 0.0361$) in 30:15 index in the treated group (1.11 ± 0.3) as compared with control group (1.21 ± 0.5). This study provides evidence that manual acupuncture in PC6 can modify the 30:15 ratio related to physiological response in subjects with type 2 diabetes. Clinical trials are needed to assess this acupuncture treatment for orthostatic dysfunction in diabetic subjects.

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Use of nutritional supplement nutrisol in the treatment of adolescents with nutritional deficiency

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Temática: Investigación, desarrollo, comercialización y marketing de productos naturales.

.Introduction: The nutritional requirements depend on the spending needed to keep the growth rhythm, changes in body composition and in the energy consumption, hence the need for an adequate nutrition for adolescents to develop their biological potential to the maximum. **Objective:** To evaluate the effectiveness and safety of Nutrisol as a nutritional supplement in the treatment of adolescents with nutritional deficiency. **Materials and Methods:** A prospective, longitudinal and analytic study at the University Pediatric Hospital William Soler was performed. 40 adolescents, 20 of each sex, with diagnosis of nutritional deficiency and percentile values equal to 10 or less were included. The teenagers were treated for a period of six months. Group I received the usual treatment (vitamin C, Polivit and dietary assessment) and group II was treated with Nutrisol and dietary assessment. Anthropometric measurements, laboratory data and clinical parameters were evaluated. The evolution of the response variables was performed using the non-parametric Friedman test. **Results:** Adolescents who consumed Nutrisol showed greater variability in the studied anthropometric variables than those receiving the usual treatment ($p = 0.001$). The exploring of haematological variables showed a slight increase of normal values for both sexes after consumption of Nutrisol. Teenage boys had better response to treatment than females. There was no change in the parameters for safety variables assessed during the study. Nausea and loss of appetite were the adverse events reported during the investigation. No serious adverse events were reported. **Conclusions:** The use of Nutrisol is effective and safe in treating adolescents with nutritional deficiencies.

Phytochemical study and evaluation of the antioxidant capacity of aerial parts of *Momordica charantia* in colombia

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Introduction

Momordica charantia (Cucurbitaceae) commonly known as a balsam, is a climbing plant, known for its native tropical hypoglycemic activity and which some important biological activities have been determined, such as anti-carcinogenic, anti-inflammatory and vasodilator (Halaweish, Tallamy & Santana, 1999). Chemical studies have reported the presence of phenolic compounds, terpenes, alkaloids, saponins and momordicosides (Raman & lau, 1996; Yadav et al., 2010). This species has been studied in Colombia, has been studied in a little way from the chemical and biological point of view, reason why this research objective was to contribute to the aerial parts of the phytochemical study and assess their antioxidant capacity.

Method

From the vegetal material collected in the municipality of Honda-Tolima (5 ° 11 '29' 'N, 74 ° 44' 36 " O) and identified in the Colombian National Herbarium under collection number COL 596915, the extract was obtained Ethanolic (4 g). For the fractionation of the extract the continuous liquid-liquid extraction method with solvents of increasing polarity was used, obtaining the fractions of heptane (1.22%), dichloromethane (8.51%) ethyl acetate (1.02%) and a hydroalcoholic residue (89.25%). Each of these fractions was subjected to continuous separations through column chromatography (gravity and medium pressure), thin layer and preparative thin layer, allowing the obtaining of three mixtures and the isolation of two saponins reported for the first time for this species in our country. The identification of the mixtures was carried out by Gas Chromatography coupled to mass spectrometry and the structural elucidation of the isolated compounds was carried out by Nuclear Magnetic Resonance (¹H, ¹³C, COSY and J-MOD).

The antioxidant capacity was determined by the DPPH and ABTS method to the extract and fractions obtained. The statistical analysis for the quantification of the antioxidant capacity (% inhibition and IC50)

was performed using the software statgraphics centurion XVI, where correlation coefficients, analysis of variance and correlation of the variables were established for each of the evaluated treatments.

Findings / Discussion / Conclusion

The separation and purification of the fractions of the aerial parts of the species *Momordica charantia* led to the isolation of two saponins identified as Kaguasaponina D and Momordicina II and three mixtures, the first composed of Epoxylinalool, Dihydroactinidiolide, palmitic acid and squalene; the second mixture formed by a bicyclic terpenoid and a norisoprenoid (3-Hydroxy-5,6-epoxy-beta-ionone and 2-Cyclohexen-1-one-4-hydroxy-3,5,6-trimethyl-4- (3 -oxo-1-butenyl)) and the third constituted by oxide linalol, cinnamic acid, benzoic acid and indole. The determination of the antioxidant capacity may establish that only the ethanolic extract and fractions of hydroalcoholic e ethyl acetate, showed an inhibition percentage greater than 40% in concentrations higher than 1000 ppm; which indicates the low antioxidant capacity of the aerial parts of *Momordica charantia* due to high concentrations in that it inhibits the radical.

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Caracterización bioquímica y evaluación de la toxicidad aguda de extractos hidroalcohólicos de cinco plantas nativas sobre *Artemia salina* Leach.

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Introducción

La explosión demográfica viene generando una necesidad de diversas estrategias para contribuir en la producción de alimentos ecológicos exentos de contaminación con plaguicidas de origen sintético, como una respuesta a la exigencia de la población, por ello se destinan fondos económicos para la investigación científica que permita encontrar plantas con potencial biocida.

Método

En el presente estudio se evaluó la toxicidad aguda del extracto hidroalcohólico de hojas de *Chenopodium ambrosioides* “paico”, *Minthostachys mollis* “muña”, *Datura stramonium* “chamico”, *Tanacetum parthenium* “santa maría”, *Senecio rudbeckiaefolia* “remilla” sobre *Artemia salina* Lech, en dosis de 10, 100 y 1000 ppm.

Resultados

Los valores de concentraciones letales en ppm para *Chenopodium ambrosioides* “paico” fue de 209,635; *Minthostachys mollis* “muña” 96,582; *Datura stramonium* “chamico” 76,175; *Tanacetum parthenium* “santa maría”; *Senecio rudbeckiaefolia* “remilla” 124,234 ppm respectivamente.

Fueron identificados los principales grupos de metabolitos secundarios presentes en el extracto mediante tamizaje fitoquímico. Los resultados obtenidos nos permiten validar la seguridad del uso tradicional del extracto hidroalcohólico de hojas de las especies en estudio, siendo los metabolitos secundarios más abundantes los alcaloides, taninos, flavonoides. La identificación taxonómica se realizó en el herbario Huamanguensis de la Facultad de Ciencias Biológicas de la Universidad Nacional de San Cristóbal de Huamanga.

Discusión



Los resultados indican que los extractos hidroalcohólicos de las especies *Chenopodium ambrosioides* “paico”, *Minthostachys mollis* “muña”, *Datura stramonium* “chamico”, *Tanacetum parthenium* “santa maría” y *Senecio rudbeckiaefolia* “remilla” tienen efecto biocida por su composición de principios bioactivos secundarios los cuales son refrendados por el porcentaje de mortalidad que presentan en cada caso, es así que las cinco especies presentan mortalidades entre un 10 a 20% para concentraciones de 10 ppm, concentraciones de 100 ppm presentan porcentajes de mortalidad en promedio de un 40%, y concentraciones de 1000 ppm entre un 80 a 90 % de mortalidad.

Conclusiones

1. Del análisis fitoquímico realizado para las hojas de *Chenopodium ambrosioides* “paico”, *Minthostachys mollis* “muña”, *Senecio rudbeckiaefolia* “remilla”, *Datura stramonium* “chamico”, *Tanacetum parthenium* “santa maría” indica la presencia de principios bioactivos como alcaloides, fenoles-taninos, triterpenos, aminoácidos.
2. La CL₅₀ de los extractos hidroalcohólicos de hojas de *Chenopodium ambrosioides* “paico”, *Minthostachys mollis* “muña”, *Senecio rudbeckiaefolia* “remilla”, *Datura stramonium* “chamico”, *Tanacetum parthenium* “santa maría” sobre *Artemia salina* Leach fueron 209,635; 96,582; 76,175; 166,103 y 124,234 ppm respectivamente.

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Effect of *Pleurotus ostreatus* powder consumption on urinary clinical parameters

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Introduction

Edible mushrooms are known for their nutritional and medicinal value. *Pleurotus ostreatus* is one of the most important species being the third one producing worldwide. *P. ostreatus* have demonstrated having health related effects such as blood glucose, cholesterol and blood pressure redactor agent, as well as anti-gastric ulcers agent, and antioxidant, antitumor, antimicrobial and immunomodulatory properties. It has been reported the traditional medicinal use of *P. ostreatus* as diuretic in some parts of Mexico and Central America. However, to the best of our knowledge there are no human evaluation studies of *P. ostreatus* diuretic properties. Thus, the aim of the present work was to evaluate the diuretic effect of dehydrated *P. ostreatus* consumption by analyzing urine parameters

Method

P. ostreatus was dehydrated at 50°C during 8 h until moisture content was less than 8% and mill to get a particle size <1 mm. The study group consisted of 30 subjects of both gender without urologic disease. *P. ostreatus* was administrated a single dose (7 g) per month during three months. A urine basal sample was collected two h previous to *P. ostreatus* consumption and was taken as control.

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Results / Discussion / Conclusion

Results showed that consumption of dried *P. ostreatus* have significant effect ($p < 0.05$) in reduction of creatinine, specific gravity and pH urine parameters. Although the mechanism of diuretic action of medicinal plants is not yet well established, it is suggested that it is due to an increase in renal circulation resulting in greater glomerular filtration. The diuretic effect of some other edible mushrooms has been attributed to their

ergosterol and D-mannitol content. In conclusion, dehydrated *Pleurotus ostreatus* food might be use as natural diuretic alternative.

Abstracts must be sent through the form on the site at the address <https://www.silae.it/habana2019/submit-abstract/>

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Modifications on blood pressure and blood variables in an intervention with the nutritional supplement Noni C® during intense exercise

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Summary

Introduction: Noni-C® is a natural product registered as a nutritional supplement, obtained from the plant *Morinda citrifolia* Lin, associated with many proven clinical qualities and traditionally used as food.

Objective: We aim to evaluate the changes in arterial pressure and blood variables in an intense physical activity supplemented with Noni-C®. **Methods:** To achieve this objective, a cross-sectional and prospective intervention study was performed in young adult patients. Two groups are formed one control and one treated, all with average ages of 23 and 24 years respectively, who were subjected to intense physical activity, for a period of two months. The measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin, hematocrit, total proteins and albumin were made; at the beginning and end of the study period. **Results:** There was a significant decrease in the SBP and DBP of the control group at the end to intense physical activity. In the supplemented group there was a non-significant increase in hemoglobin and hematocrit, and a significant increase in total proteins and albumin. **Conclusions:** The intervention with the nutritional supplement Noni-C® produced favorable modifications in some of the blood parameters studied.

Keywords: Noni, systolic artery pressure, hemoglobin, hematocrit, total proteins, albumin, physical activity.

Chemical composition, acute toxicity and hemolytic effects of the species *Scabiosa stellata*

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Introduction

The use of natural compounds isolated from medicinal plants for the treatment of various diseases is one of the strategies applied to find new drugs with few side effects. However, some plants could induce toxic symptoms and hemolytic anemia. In this context, we are interested in the chemical investigation and the evaluation of acute toxicity and hemolytic activity of the *n*-butanolic extract obtained from the medicinal plant *Scabiosa stellata* L. belonging to the family Dipsacaceae growing in Algeria. It is distributed in North Africa and used in folk medicine for the treatment of many human diseases as an expectorant, purifying, diaphoretic, stomachic, appetizer and digestive. It is also indicated in cases of bronchitis, bronchial pneumonia, influenza and asthma, and against cracks in the heel.

Method

The multistep chromatographic separations and purifications (VLC, CC and TLC) were applied on *n*-BuOH extract of the aerial parts of *S. stellata* allowing the isolation of two saponins. Their structures were established by spectroscopic techniques including 1D and 2D NMR (¹H, ¹³C, DEPT, COSY, HSQC, HMBC, TOCSY and NOESY) and ESI-MS, and by comparison with the literature data. The cytotoxicity of extracts was tested by Brine shrimp lethality method and the hemolytic activity was assessed by spectrophotometric method towards human erythrocytes.

Results / Discussion / Conclusion

The chemical study of *n*-BuOH extract of *S. stellata* led to the isolation and structural elucidation of two glycosylated triterpenes namely Hederagenine 3-O-[β-D-xylopyranosyl-(1'''→3'')-O-α-L-rhamnopyranosyl-(1''→2')-O-α-L-arabinopyranosyl] (**1**) and Hederagenine 3-O-[β-D-glucopyranosyl-(1'''→4''')-β-D-xylopyranosyl-(1'''→3'')-O-α-L-rhamnopyranosyl-(1''→2')-O-α-L-arabinopyranosyl] (**2**). The structures of these saponins were determined by 1D and 2D NMR (¹H, ¹³C, DEPT, COSY, HSQC, HMBC, TOCSY and NOESY) and ESI-MS, and by comparison with the literature data. The acute oral toxicity was evaluated on albino Wistar rats, according to the OECD guideline 423 and the hemolytic activity was assessed spectrophotometrically towards human erythrocytes. The oral administration of *n*-BuOH extract at various

concentrations (500, 1000 and 2000 mg/kg) induce toxic symptoms and mortality of all the treated animals with the dose of 2000 mg/kg. A significant increase in biochemical parameters including (ALT, AST, cholesterol, triglycerides, creatinine, and urea) and important alterations in the hematological parameters compared to the control group were observed in both survived groups (500 and 1000 mg/kg). The histological examination of the liver, kidney, and lungs confirmed the observed toxic signs. Therefore, the approximate acute lethal dose 50 was estimated to be higher than 500 mg/kg. The hemolytic assay exhibited that n-butanolic extract induces hemolysis in a dose-dependent manner with a value of EC_{50} at 37.3 ± 0.1 $\mu\text{g/mL}$. The hemolytic and toxicity effects seem to be related to the presence of saponins. Furthermore, it could be concluded that a prudent use in traditional medicine of the species *S. stellata* is recommended in order to get a safe therapeutic use.

Inhibition *in vitro* HMG - CoA reductase and antioxidant activity of the extract methanolic of *Jatropha gossypifolia*

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Introduction: The *Jatropha gossypifolia* is a plant, used etnofarmacologicamente for treating certain conditions such as diabetes, hypertension and hypercholesterolemia, which is why it is considered important to evaluate the antioxidant activity and determining the ability of inhibiting the enzyme HMG Co A reductase, drug targets in the treatment of hypercholesterolemia and arterial hypertension. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG- CoA) was evaluated.

Method The inhibition of HMG- CoA, and antioxidant activity of *Jatropha Gossypifolia* methanol extract was evaluated. The HMG- CoA reductase assay kit (Sigma, St. Louis, USA; Catalog number CS1090) was used, which contains the human catalytic domain of the enzyme HMGR (concentration 0.6 mg protein / mL) and pravastatin as a positive control. To evaluate the antioxidant activity, the method proposed by Grinberg was used, 100 µL human erythrocyte content / mL was incubated and the *Jatropha Gossypifolia* extract was administered and the supernatant was used to measure the released hemoglobin.

A 100% red cell hemolysis blank was taken as a negative control at different concentrations at a temperature of 37 ° C under constant stirring for 30 minutes in the presence of 5% H₂O₂ (v / v). The results are shown as the mean ± standard deviation (EE); they were processed using GraphPad Prism (version 5.00 for Windows). The differences between the concentrations of the analyzed extracts were evaluated by means of the ANOVA test and for all the analysis the significance criterion was established at p <0.05.

Results / Discussion / Conclusion: The concentrations chosen to carry out the tests were: 0.0001, 0.001, 0.01 and 0.1 ppm, this was determined prior to a leukocyte cytotoxicity test performed by the trypan blue exclusion method. The extract concentrations inhibits the enzyme HMG- CoA reductase at different levels, especially at the concentration of 0.001 ppm, where it inhibits around 76.5%, it could be associated with components of low to medium polarity mainly, it was also shown that a concentration of 0.1 ppm the extract inhibits 96.6% of hemolysis in human erythrocytes induced by H₂O₂ with a p <0.001

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Actividad antitumoral y estudio químico del aceite microencapsulado de semillas de *Cucurbita pepo*

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Introducción

Al aceite de semillas de *Cucurbita pepo* es utilizado con éxito en la prevención y el alivio de los problemas de próstata y de la vejiga¹⁻². Sin embargo, a pesar del amplio uso que tiene en la población los estudios científicos como quimiosensibilizador en la terapia contra el cáncer, en Cuba, son escasos. En este estudio se evaluaron física y químicamente el aceite microencapsulado y se evaluó su efecto antitumoral y quimiosensibilizador combinado con citostáticos.

Método

El aceite microencapsulado fue evaluado mediante sus perfiles CCD, CLAR, CG. Las células CT26 y los ratones a los cuales se le indujo metástasis pulmonal fueron tratados con el aceite microencapsulado en un amplio rango de concentraciones y fueron seleccionadas las dosis subefectivas para utilizarla en el tratamiento combinado con citostáticos de platino y 5-fluorouracilo, usando diferentes esquemas de tratamiento.

Resultados / Discusión / Conclusión

Los perfiles CCD, HPLC y CG mostraron la presencia de cucurbitacinas y ácidos grasos como compuestos mayoritarios. El aceite microencapsulado no fue citotóxico para las células CT26 y su combinación con

cisplatino, oxaliplatino, carboplatino y 5-fluorouracilo fueron más efectivas con respecto a los citostáticos. El mejor efecto quimiosensibilizador in vitro fue logrado con la combinación simultánea con oxaliplatino. El aceite microencapsulado mostró un efecto antimetastásico en relación directa con el incremento de su concentración. Su mejor efecto quimiosensibilizador in vivo fue obtenido en el pretratamiento combinado secuencial con oxaliplatino y con la combinación simultánea de oxaliplatino con 5-fluorouracilo. Los resultados obtenidos demuestran el potencial antitumoral del aceite de microencapsulado de semillas de *Cucurbita pepo*.

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Estudios toxicológicos preclínicos del extracto acuoso de las flores de *Kigelia africana*(BENTH)

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Cuba posee una flora muy rica y diversa de la cual se conoce que una cantidad de estas han sido introducidas, ejemplo de ello lo es *Kigelia africana* (Lam.) Benth. Es una especie arbórea endémica de África y que en la actualidad es potencialmente invasora en nuestro país. El estudio de esta planta está cobrando gran interés nacional ya que los experimentos sobre el efecto de sus extractos y de algunos de sus compuestos puros han corroborado sus propiedades medicinales, por tales razones el aprovechamiento de sus actividades terapéuticas representan un campo cuyo mercado evidencia perspectivas halagüeñas. Se han reportado efectos toxicológicos de los diferentes extractos de la fruta, corteza del tallo y hoja pero no de sus flores por lo que la presente investigación tiene como objetivo: Evaluar aspectos toxicológicos que involucran al sistema inmune posteriores a la administración de un extracto acuoso de *Kigelia africana* en ratones Balb/c. La investigación incluyó una evaluación toxicológica del producto, mediante un estudio de toxicidad aguda a dosis fija (Test de las clases) de 2000, 1000, 500 y 300 mg/kg de p.v en ratones Balb/c. Se determinó adicionalmente su potencial inmunotóxico a dosis de 50, 100 y 200 mg/kg de p.v mediante las técnicas de proliferación celular e índice mitótico (metafase) en órganos linfoides a las 24 y 48 horas de administrado el producto en los animales. Además se demostró la pertinencia del medio de cultivo Hank's como nutriente celular adecuado en la viabilidad celular. Los resultados de los estudios de toxicidad mostraron una relación dosis efecto ya que el producto en dosis bajas no evidenció signos de toxicidad no siendo así en dosis de 1000 y 2000 mg/kg, en donde se presentaron diferentes signos de toxicidad aguda en los animales (letargo, somnolencia y piloerección) y los hallazgos macroscópicos durante la necropsia mostraron rigidez muscular, deshidratación, riñones tumefactos y corazón en sístole. Por lo que el producto se clasificó según la OECD en la categoría 4. El medio Hank's empleado respondió eficientemente con una duración estable de su pH en el tiempo de la investigación, además los índices de viabilidad celular se encontraban dentro de los parámetros establecidos para su uso. Por otra parte el índice de linfocitos en metafase se comportó de forma normal y permite inferir que los niveles posológicos investigados se aceptan de forma preliminar. El presente trabajo demostró que el extracto acuoso de las flores de *Kigelia africana* en las dosis evaluadas no provocó alteraciones significativas en el sistema inmune, sin embargo deben tomarse

con precaución cuando se trata de su uso terapéutico ya que se necesitan análisis más profundos antes de poder llegar a conclusiones finales.

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Hypoglycemic activity of the hydroalcoholic extract of the bark of *Vallea stipularis* L.f. "ccenccoy" in Holtzman strain rats

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Introduction

Predictions made by WHO indicate that global growth in the prevalence of mostly type 2 diabetic patients will reach 439 million people by 2030. The use of plants with medicinal benefits represents an option, such as the *Vallea stipularis* with hypoglycemic activity and become an economic alternative for producers in the region.

Method

Basic experimental research with DCA. The sample was collected in the district of Churcampa-Huancavelica, and subjected to hydroalcoholic extraction. The hypoglycemic activity was determined using the method of hyperglycemia induced with aloxane in rats according to Kameswara Rao et al., (1999). Using 35 male Holtzman strain rats distributed in seven groups with weight between 180 - 210 g, the target received distilled water 2 ml/kg of weight, the control aloxane 180 mg/kg, the standard glibenclamide 5 mg/kg, and insulin 4 UI/kg. The hydroalcoholic extract of *Vallea stipularis* L.f. was administered to the experimental group at doses of 100, 200 and 400 mg/kg.

Results

The phytochemical march according to Lock de Ugaz, shows presence of flavonoids, tannins, polyphenols, reducing sugars, anthocyanins and catechins. The hydroalcoholic extract of the bark of *Vallea stipularis* L.f. "ccenccoy" exerts a hypoglycemic effect, the dose of 400 mg/kg is statistically significant to glibenclamide. Thin layer chromatography shows that flavonoids are present in the ethyl aceto fraction and the chloroform fraction.

Discussion

The phytochemical march of the bark of *Vallea stipularis* L.f. shows the presence of flavonoids and phenolic compounds similar to that pointed out by Chaparro et al. (2016). Rivera in 2018, through Tukey's test found that glibenclamide and phenolic compounds are statistically similar, and that phenolic compounds isolated from the rhizomes of *Zingiber officinale* Roscoe have hypoglycemic effect. The comparative effect of



glibenclamide and insulin do not differ much between them. At a dose of 100 mg/kg of the extract there is no significant activity. On the other hand, the doses of 200 and 400 mg/kg do show therapeutic activity. Kwon et al. concluded that tyrosine kinase blockade is another mechanism by which quercetin has effects against diabetes.

Conclusions

The hydroalcoholic extract at 80% of the bark of *Vallea stipularis* L.f. "ccencocoy" has hypoglycemic effect, with greater percentage of effectiveness the dose of 400 mg/kg statistically significant.

The secondary metabolites present in the hydroalcoholic extract of the bark of *Vallea stipularis* L.f. are mainly flavonoids, phenols and cardiotoxic glucosides.

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Antimicrobial effect of the aqueous extract of dormilone flower (*Mimosa albida*) in bacterial strains (*Escherichia coli* 25922, *Escherichia coli* 11229, *Pseudomonas aeruginosa* and *Staphylococcus aureus*)

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Introduction

In recent years the study of medicinal plants has been taken up again, in search of new active principles in response to the need to achieve effective alternatives for the control of bacterial infections, one of these plants is “dormilona” (*Mimosa albida*) originating in America is a medicinal plant whose properties, ranging from soothing a pain to serving as a relaxant, normally use its leaves and root, from the latter an aqueous extract was obtained which was evaluated in bacterial strains of *Escherichia coli* 25922, *Escherichia coli* 11229, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, to identify its efficacy as an antimicrobial.

Method

We used the disk diffusion methodology or Kirby Bauer method, the antibiogram consists of depositing in the agar surface, previously inoculated with the microorganism of each strain, sensitisks that were impregnated in the different antibiotics as positive controls; nitrofurantoin for *Escherichia coli* 11229, *Escherichia coli* 25922, *Pseudomonas aeruginosa* and *ceftriaxone* for *Staphylococcus aureus*, 5 dilutions of the aqueous extract of the *Mimosa albida* plant were made, after 16-18 hours of incubation at 37 ° of temperature, the sensidiscs appear surrounded by a zone of inhibition, which are read by measuring the radius of the halos where the case of each test, each test was performed in triplicate.

Results / Discussion / Conclusion

The results that were obtained in the antibiograms, forming haloes of inhibition for the five concentrations of the extract of the *Mimosa albida* plant of 300, 400, 500, 600 and 700 µg / µL expressing greater sensitivity in the strain *Escherichia coli* 11229 in the concentration 300 µg / µL with a radius of 11.56 mm compared to the inhibition zones in the strain *Staphylococcus aureus*, which presented in the concentration 300 µg / µL with a radius of 2.51 mm, very noticeable inhibition zones were obtained in the positive controls where it

was applied the specific antibiotic for each strain; *Escherichia coli* 11229 with a halo of inhibition of 12mm, *Escherichia coli* 259922 radius of 7.27 mm and *Pseudomona aeruginosa* radius of 5.56 mm compared to Nitrofurantoin, *Staphylococcus aureus* radius of 15.12 mm with Ceftriaxone on the other hand in the negative control sterile water, did not present halos of inhibition in any strain.

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Analysis of the aqueous extract of suspó flower (*Pseudobombax ellipticum*) as an antimicrobial

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Introduction

Plants have been used for a long time to treat various diseases. The growing demand for medicines based on medicinal plants, has made its study one of the new alternatives of science to determine its pharmacological properties. The objective of the present investigation was to evaluate the antimicrobial activity of the aqueous extract of suspó flower (*Pseudobombax ellipticum*).

Method

The antimicrobial test for the extract was carried out using the Kirby-Bauer method, with the following strains: *Escherichia coli* 11229 and 25229, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

In MacConkey culture medium, once measured with the spectrophotometer according to the strain its OD (Optical Density) ranging from 700 to 900 nm depending on the strain, the inoculum was extended with a swab, the discs were previously placed: control negative (-) water, positive control (+) antibiotic that was according to its nitrofurantoin and / or ceftriaxone strain. The concentrations used of the extract were 300 µg / µL, 400 µg / µL, 500 µg / µL, 600 µg / µL and 700 µg / µL.

Subsequently, they were incubated from 16-18 hrs at 37 ° C

Results / Discussion / Conclusion

Once the incubation time had been reached, the zone of inhibition was measured to perform the statistical analysis, according to the data obtained, the following conclusion is reached where the aqueous extract of the suspó flower (*Pseudobombax ellipticum*) has greater inhibition activity. the following strains *Escherichia coli* 25229 when it has a concentration of 300 µg / µL with an average halo of 7.19 mm and *Staphylococcus aureus* in the concentration of 400 µg / µL with an average halo of 5.78 mm.

In the strain of *Pseudomonas aeruginosa* and *Escherichia coli* 11229 the expected results were not obtained since their inhibition behavior is minimal.

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Biocide effect of saponins of *Chenopodium quinoa* Willd on larvae of *Phthorimaea operculella*. Ayacucho

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Introduction

Natural products of vegetable origin with insecticide activity are valid alternatives for pest control in substitution of conventional synthetic pesticides, since they do not generate resistance, undesirable effects on organisms and negative impacts on the environment.

Method

The study was basic with experimental design. The extraction of yellow quinoa saponins, quantification by spectrophotometry, phytochemical screening, evaluation of the biocidal effect and the mean lethal concentration were performed. Saponin solutions of 1, 2.5 and 5% (3 repetitions and their control in a DCA) were used. Larval mortality was evaluated at 8, 19, 28 and 38 days of exposure. For pathogenicity, 20 potato moth postures were used.

Results

At 155.4 µg saponin a 27% mortality of larvae was observed; at 388.5 µg 36%; at 1368.5 µg 30%; the mean lethal concentration (LC50) on larvae of *Phthorimaea operculella* was 13583.4 µg saponin, which allowed to know its biocidal effect, when increasing the concentration the percentage of mortality was also increased, but this difference was not significant ($p > 0.05$, $\alpha = 0.05$). Phytochemical screening of quinoa seeds showed that saponins and triterpenes are the most abundant.

Discussion

The extraction of saponins was carried out in 50% ethanolic solution and controlled temperature in the microwave (78°C); data coinciding with Zarate (2016), who used 50% v/v ethanolic solution at 78.4°C when extracting saponins from different varieties of quinoa. Bonifaz evaluated the insecticidal activity of hydrolyzed and non-hydrolyzed quinoa saponine on *Drosophila melanogaster*, finding positive results of the biocidal action of hydrolyzed quinoa saponins on *Drosophila*, and also reports several plants capable of

synthesizing secondary metabolites that have biological properties of great importance against pest insects (Bonifaz, 2010); results that when compared with those obtained in the work have some similarity, since no significant differences were found with the concentrations worked.

Conclusions

The main active metabolites of the plant are saponins and triterpenes, the toxic effect of quinoa seeds is attributed to saponins of the triterpenoid type. The LC₅₀ of the saponins of *Chenopodium quinoa* Willd against larvae of *Phthorimaea operculella* was 13583.4 µg of saponin, which allowed to know its biocidal effect.

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Capacidad fotoprotectora del extracto de *Baccharis antioquiensis* y su potencial aplicación en protectores

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Introducción

Un aumento considerable y preocupante, del cáncer de piel, se ha venido observando debido a la exposición permanente de las personas a la radiación ultravioleta (UVR), ocasionando diversas patologías tales como eritema, fotoenvejecimiento, entre otras (de Gruijl et al., 1999). Aunque se han desarrollado protectores solares cosméticos para proteger la piel expuesta, los cuales contienen filtros físicos y químicos, los últimos han evidenciado problemas de fotodegradación, absorción sistémica, pérdida de eficacia y posibles efectos tóxicos locales y sistémicos. Así, se hace necesario estudiar nuevas posibilidades de filtros solares de origen natural con las características óptimas de fotoprotección de amplio espectro (Serpone et al., 2007). En este sentido, se considera una fuente importante de ellos las plantas de ecosistemas de alta montaña; estas especies están expuestas a una intensa radiación solar y bajas temperaturas, que las ha llevado a generar mecanismos de defensa para mantener la homeostasis y así sobrevivir a las condiciones de estrés ambiental a las que está expuesta este tipo de vegetación. Esto lo han logrado a través de la producción de metabolitos secundarios como polifenoles, que poseen actividades antioxidantes y fotoprotectoras; siendo una de dichas plantas la especie *Baccharis antioquiensis* (Chen et al., 2012; Mejía Giraldo et al., 2016).

Por consiguiente, se pretende determinar la capacidad de fotoprotección y fotoestabilidad del extracto hidrolizado de *B. antioquiensis* para emplearse como un fotoprotector.

Método

El material vegetal se secó a temperatura ambiente, se molió y desengrasó para hacer un extracto metanólico, el sobrenadante fue tratado con carbón activado, posteriormente filtrado y concentrado. Luego se sometió a un proceso de hidrólisis ácida en reflujo y extracción con acetato de etilo. Se realizaron caracterizaciones del extracto hidrolizado mediante HPLC e IR; los ensayos de capacidad antioxidante con el método de DPPH, contenido total de polifenoles (TPC) se determinó con el reactivo de Folin y la capacidad de fotoprotección y fotoestabilidad se evaluó *in vitro* mediante espectrofotómetro de transmitancia difusa.

Resultados / Discusión / Conclusión

El extracto de *Baccharis antioquiensis* tiene potencial como fotoprotector de amplio espectro, con coeficientes de absorción ((Absorbancia)/(mg del extracto seco/mL)) de $12,4 \pm 0,8$ en 290 nm, $13,4 \pm 0,9$ en 310 nm, $14,7 \pm 0,8$ en 340 nm y $2,8 \pm 0,2$ en 380 nm comprobando su fotoprotección en el UVB-UVA; además presenta capacidad antirradicalaria *in vitro* expresada como EC_{50} de 0,1395 comparada con butil hidroxitolueno con EC_{50} de 0,1300 esta actividad antioxidante produce un efecto aditivo en la fotoprotección, y es debido principalmente a la presencia de polifenoles donde *B. antioquiensis* tiene un contenido de polifenoles totales de $143,88 \pm 3,3$ (mg equivalentes de ácido gálico/g material vegetal seco). Demostrando el potencial de los compuestos de origen natural con actividad de fotoprotección en la formulación de protectores solares.

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Hypolipidemic and anti-inflammatory activity of metabolites extracted from *Agave brittoniana* subsp. *Brachypus*

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Abstract

Introduction: Saponins and their steroidal saponinins are considered responsible for different biological effects. The *Agave brittoniana* subsp. *brachypus* presents mainly this type of metabolites, including yucagenin and diosgenin, the latter with numerous corroborated pharmacological properties. **Objectives:** To verify the pharmacological potential of the species in experimental models of hyperlipidemia and inflammation. **Materials and Methods:** In vivo protocols were designed to evaluate the pharmacological effect of saponins and / or saponinins, or fractions rich in yucagenin, in a chronic model of metabolic syndrome, acute induction of hyperlipidemia, as well as acute and chronic models of systemic and intestinal inflammation. Studies of acute toxicity and genotoxicity were also designed. **Results:** Hydroalcoholic and crude saponin extract showed antiobesity activity in a chronic hyperlipidemia model, with a decrease in body weight, visceral adiposity and triglyceride values. Saponins and saponinins are able to reduce lipid elevation at 48 hours post induction in the acute hyperlipidemia model. In both acute and chronic inflammation models, the pharmacological effectiveness of crude saponins, saponinins and a fraction rich in yucagenin was demonstrated. In models of ulcerative colitis, an anti-inflammatory and cicatrizing effect of saponins of the species were demonstrated. There were no signs of toxicity in acute studies, nor genotoxic effect at the doses tested. **Conclusions:** The steroidal metabolites extracted from the species have a marked lipid-lowering and anti-inflammatory effect, as well as the absence of signs of acute exposure toxicity, which makes them an attractive proposal for the development of pharmaceutical formulation of plant origin.

Regulatory Framework of Traditional, Complementary and Integrative Medicine in Cuba

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In the last decade, there has been a global upsurge in the use of traditional medicine and complementary and alternative medicine in both developed and developing countries. This is one of the main reasons for reinforcing the surveillance of the safety, efficacy and quality control of traditional medicine, complementary and alternative medicines.

This work describes important aspects about the art state of the regulatory status of herbal medicines. Besides that, data related with the countries involved in the World Health Organization (WHO) program for traditional medicine will be showed.

Besides that, it *shows* the characteristics of the traditional medicine in Cuba and the main requirements for the registering of herbal medicinal products in Cuba. The market and the main challenges are analysed in the investigation of the phytomedicines as well as the tendencies in the growth of this attractive sector. The strategies for the development of herbal medicinal products in Cuba are showed as well as some of the interactions between natural and synthetic drugs in Cuba as a part of Pharmacovigilance Program.

Drug Regulatory Authorities should ensure the quality, safety and efficacy of traditional medicines.



Effect of gestational exposure to *Ibervillea sonorae* on reproductive parameters of mice

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Introduction

The use of medicinal herbs during pregnancy is common worldwide and aims to treat various symptoms and / or diseases of pregnancy. *Ibervillea sonorae* or Wereke is a species native of northwestern Mexico (1). In the last decade, its consumption has increased as a therapeutic alternative to reduce the blood glucose levels of people with type 2 diabetes mellitus (2); However, although there have been several investigations of the efficacy of this root as hypoglycaemic (3), there is no information available on the safety of its consumption during pregnancy. The purpose of this work was to evaluate the effects of the administration at increasing doses of the Wereke lyophilized aqueous extract to pregnant mice, in order to determine the concentration that affects reproductive parameters.

Method

Pregnant ICR-CD1 mice were used and were randomly assigned to six groups: Control and Wereke in doses of 200, 300, 450, 675 and 1013 mg / kg. Wereke's lyophilized aqueous extract was administered intragastrically from day 5 to day 17 of gestation. The weight gain of the pregnant females was recorded on days 0, 6, 10, 15 and 18 of the gestation (GD), and a blood sample was taken from the caudal vein on days 0, 6, 12 and 18 GD to determine blood glucose. On the 18th day of pregnancy, animals were sacrificed and hysterectomized to record the number of corpora lutea, implants, live and dead fetuses, placentas, early and late reabsorption. In addition, the size and weight of both placentas and fetuses were determined.

Results / Discussion / Conclusion

The results of this investigation showed that the intragastric administration of the lyophilized aqueous extract of Wereke exerts hypoglycaemic activity when administered during pregnancy to mice from 450 mg / kg dose and above. However, this same dose of extract causes embryoletality, and at a dose of 1013 mg / kg produces fetotoxicity manifested as a decrease in fetal weight. Other studies have reported that other species

of the Cucurbitaceae family exhibit anti-fertility and anti-implantation activities, which may be related to their cucurbitacin content (4). Thus, it is concluded that exposure to the lyophilized aqueous extract of *Ibervillea sonora* during the gestation of ICR-CD1 mice affects its progeny, both in the embryonic and fetal stages. It is necessary to continue with the evaluation of the root in other rodent and non-rodent models, as stipulated by international regulatory agencies and, in turn, caution is advised in its use by pregnant women.

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Desarrollo de un nuevo método analítico para cuantificar hemo en ANT-00

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Introducción

ANT-00 es una formulación de origen natural que contiene miel de abejas, sangre bovina, extracto acuoso abomasal, benzoato de sodio y propóleos. El mismo tiene como compuesto activo de mayor importancia farmacológica la molécula de hemo, que aporta la hemoglobina de la sangre bovina. BioCen está trabajando en la obtención de un nuevo medicamento en forma sólida que contenga ANT-00 como Ingrediente Farmacéutico Activo. El objetivo de este trabajo fue desarrollar y validar el método de cuantificación de hemo en ANT-00.

Método

El método consistió en la solubilización del hemo en una disolución de NaOH 0,1 M y Tritón X-100 al 2,5 %. La concentración de hemo se determinó espectrofotométricamente a partir de la absorbancia (575 nm) de la muestra y un patrón de trabajo de hematina porcina. Se determinó la influencia de dos concentraciones de NaOH (0,1 M y 0,5 M) en el disolvente así como la influencia de la centrifugación en tres lotes diferentes. Se calculó el coeficiente de extinción molar de la hematina porcina en las condiciones de trabajo y se realizó un estudio de estabilidad durante 1 mes, a temperatura de refrigeración y a temperatura ambiente, de tres lotes del patrón de trabajo a la concentración de 60 mg/mL. Se estudiaron los parámetros de especificidad, linealidad, exactitud y precisión.

Resultados/Discusión/

Se obtuvo como resultado que no existen diferencias significativas entre una u otra concentración de NaOH ($P=0,0654$) ni entre las muestras centrifugadas o no ($P=0,1660$; $P=0,6803$; $P=0,9311$). El coeficiente de extinción molar de la hematina porcina obtenido fue de $0,645 \pm 0,303$ L/cm.mol. En el estudio de estabilidad se obtuvo como resultado que el patrón es estable a ambas temperaturas utilizando como criterio de aceptación que el Coeficiente de Variación entre los valores de absorbancia fuera menor del 5%. El método además mostró ser específico, lineal, exacto y preciso, al cumplir con los criterios de aceptación requeridos. Estos resultados nos permiten continuar con el desarrollo del nuevo medicamento en forma sólida.

Effect of the gestational exposure to *Ibervillea sonorae* on fetal morphology of mice

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Introduction

Congenital malformations affect 1 in 33 infants, cause 3.2 million disabilities per year and cause the death of 276,000 newborns during the first weeks of life (1). Traditional medicine has been used for thousands of years for its contribution to health, forming part of the culture of the people, including the pregnant woman, since it is assumed that, due to its natural origin, it is harmless. The *Ibervillea sonorae* (Wereke), is native to the Sonoran desert, Mexico. It is used to relieve skin conditions, arthritis, diabetes, rheumatism and heart disease; and antibiotic, antifungal and antiviral properties are also attributed to it (2). Despite being a free sale, no safety studies have been carried out regarding its teratogenic potential. Therefore, the purpose of this work was to evaluate the effect of gestational exposure to *Ibervillea sonorae* (Wereke) on the fetal morphology of mice.

Method

Pregnant ICR-CD1 mice were used, which were randomly assigned to six groups: Control and Wereke at doses of 200, 300, 450, 675 and 1013 mg/kg. The lyophilized aqueous extract of Wereke was administered intragastrically from day 5 to day 17 of gestation. On day 18 of pregnancy, females were sacrificed by cervical dislocation and a hysterectomy was performed. In accordance with OECD Guide 414, its dysmorphic potential was determined using a stereomicroscope to detect possible external fetal malformations. Finally, fetuses were processed to register the possible visceral and skeletal malformations. The analysis of the skeletal abnormalities was carried out with one third of each litter using the Peters bichromatic technique. The remaining two thirds were processed to determine visceral malformations by Wilson razor blade slices method.

Results / Discussion / Conclusion

The results indicated absence of external malformations, moderate hydronephrosis, grayish coloration of kidneys that suggests necrotic processes, and the appearance of an extra rudimentary rib at all doses. The administration of 450, 675 and 1013 mg / kg caused an increase in the ossification of cranial bones (frontal, parietal, interparietal and nasal bone) in the progeny of ICR-CR1 mice exposed during pregnancy. Thus, despite exposure to Wereke during intrauterine development did not produce external malformations, it altered processes related to nephrogenesis and osteogenesis, from doses that were used in other studies for pharmacological purposes (4, 5), which could be related to its content of polyphenols, which, although they have antioxidant activity, can also act as pro-oxidants (6), such as vitamins C and E; or to other mechanisms. Conclusion: Due to the production of hydronephrosis at therapeutic doses of Wereke *per se*, and to the nature of other findings found in this work, it is suggested to continue with the study of the teratogenic potential of the *Ibervillea sonorae* root in other species, as indicated by the international regulatory bodies.

This study was supported by the Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (Project No. 20172029).

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Identification of secondary metabolites of two species of Capparaceae family in southern Ecuador

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Introduction

The vegetal diversity and its wide cultural richness constitute from remote times a valuable resource to cure or alleviate diseases. The flora rich in metabolites that is found in our country has favored the value of plants especially for their phyto-therapeutic properties found in extracts or in their active ingredients (Bermúdez, Oliveira, & Velázquez, 2005). Currently in Ecuador there are a large number of medicinal plants without having undergone a phytochemical study such as those belonging to the Capparaceae family, and which however have a medicinal utility in different parts of the country (Torre, Muriel & Balslev, 2006).

The Capparaceae it has 39 genera and 650 species that are distributed mainly in warm regions around the world, there are known for their high nutritional value and therapeutic properties. (Gull, Anwar, Sultana, Alcayde, & Nouman, 2015). Species *Cynophalla mollis* and *Colicodendron scabridum* have been related to bioactive molecules, such as natural antioxidants (flavonoids), alkaloids, terpenoids, vitamins and antimicrobial agents that give interest activities cardiovascular, antimicrobial, anti-inflammatory, antirheumatic, antihypertensive, hepatoprotective and hypoglycaemic that are attributed to different parts of the plant (Mollica et al., 2019).

Method

The aerial parts of *Cynophalla mollis* (Kunth) J. Presl and *Colicodendron scabridum* (Kunth) Seem were collected in Loja city, canton Catamayo. The selected leaves were subjected a drying process at 34°C and weighed 500g to macerate with solvents of different polarities for obtained the total extracts (hexane, acetate, methanol). Each extract was subjected to fractionation by column chromatography using silica gel as the fixed phase. The isolated compounds were identified by spectroscopic techniques such as NMR, CG-MS, the results were compared with literature.

Results

Table 1. Secondary metabolites isolated

Extracts	Hexane	Acetate	Methanol
<i>Cynophalla mollis</i>		Terpenes	Flavonoid Alkaloides
<i>Colicodendron scabridum</i>	Terpenes	Terpenes	Flavonol Alkaloides

Conclusion

Seven molecules were identified in the *species C. scabridum*, 4 terpenes, 2 alkaloids of pyrrolidinic origin, and 1 glycosylated flavonol. While from *C. mollis* obtained 6 molecules, 4 terpenes, 1 pyrrolidinic alkaloid, and 1 glycosylated flavonoid.

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Evaluación de la toxicidad aguda del extracto acuoso y etanólico de hojas de *Salvia urica* en rata y *Artemia franciscana*

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Introducción

Los productos naturales son un tratamiento primario para una gran variedad de enfermedades, por lo tanto, las plantas medicinales tienen un papel importante en la búsqueda y desarrollo de nuevos fármacos. El género *Salvia* L., se encuentra dentro de la familia *Lamiaceae*, destaca por su diversidad y endemismo en México, con alrededor de 300 especies. Las especies aromáticas de este género han sido utilizadas tradicionalmente por sus diferentes propiedades medicinales y terapéuticas. *Salvia urica* es una planta aromática, herbácea nativa de zonas montañosas de México; esta planta es usada tradicionalmente para el tratamiento de la diabetes mellitus. Por ello es necesario realizar evaluaciones de eficacia farmacológica y toxicidad que permita usar la planta de manera segura.

Método

La planta *Salvia urica* fue colectada en la localidad de Santiago Xihuitlán, Juchique de Ferrer Veracruz México, algunos ejemplares fueron colectados y llevados para su autenticación con personal especializado. Una vez obtenidos los extractos acuoso y etanólico de hojas de *Salvia urica* se realizaron pruebas coloridas (Domínguez, 1979), esto con el objetivo de identificar las familias de metabolitos presentes. Por otra parte, para la evaluación de toxicidad aguda, primero se realizó un bioensayo con *Artemia franciscana*, el cual consistió en eclosionar los quistes de acuerdo con la norma mexicana NMX-AA-110-1995-SCF, una vez eclosionados, los metanauplios, se colocaron en grupos de 10 en 2 mL de agua de mar artificial previamente oxigenada y se agregaron concentraciones crecientes de los extractos de interés (1,000, 10,000, 100,000 y



1,000,000 µg/ml). Los individuos fueron mantenidos 24 h en condiciones estándar y se determinó el porcentaje de muerte, con los resultados obtenidos se realizaron los cálculos correspondientes para determinar la concentración tóxica. En un segundo experimento y de acuerdo al esquema de Lorke (1983), se emplearon ratas macho adultas que en una primera fase recibieron diferentes dosis de los extractos (10, 100 y 1000 mg/Kg) y se observaron durante 14 días, una vez que se verificó que no murió ninguno se procedió a una segunda etapa, en la que se ensayaron las dosis de 1600, 2900 y 5000 mg/Kg.

Resultados / Discusión / Conclusión

Las familias de metabolitos encontradas fueron alcaloides, flavonoides, saponinas, cumarinas y terpenos. En el bioensayo con *Artemia franciscana*, el porcentaje máximo de mortalidad fue del 90% para el extracto etanólico ($CL_{50}=241,124 \mu\text{g/mL}$) y 83% para el acuoso ($CL_{50}=278,786 \mu\text{g/mL}$). En otras plantas se ha relacionado su toxicidad con la presencia de aceites esenciales o terpenos, estos último los encontramos en el extracto etanólico, lo cual puede explicar el mayor porcentaje de mortalidad. En cuanto al esquema de Lorke, ninguna de las dosis ensayadas de los extractos acuoso y etanólico de *Salvia urica*, provocaron la muerte de los animales, la dosis tóxica de ambos extractos es superior a 5000 mg/Kg. Por lo tanto, los extractos ensayados pueden ser considerados seguros. Estos resultados son similares a los obtenidos con otras plantas de uso tradicional tales como *Picrasma crenata* y *Alternanthera halimifolia*, las cuales ejercen efectos hipoglucemiantes y carecen de efectos de tóxicos.

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Aflatoxin B1 impact on breast cancer cell lines

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Introduction

Aflatoxin B1 (AFB1) is a widespread mycotoxin causing concern for its carcinogenicity: indeed, it has been indicated as a Group 1 human carcinogen by IARC [1], with several studies reporting an association with different cancers development [2]. Few data are present on the effect of AFB1 on breast cancer [3]. It is known that AFB1 carcinogenicity is via CYP450-mediated activation, enzymes expressed in breast tissues. Since mammary tissues are a route through which AFB1 or its metabolites can be secreted in milk [2] and considering the poor amount of available studies, aim of this work is to explore the effects of AFB1 on a panel of breast cancer cell lines.

Method

Three breast cancer cell lines, MCF-7 (ER+), SKBR3 (HER2+) and MDA-MB-468 (triple negative), were used. Cell viability was assessed by colorimetric sulforhodamine B assay (SRB, Sigma Aldrich): cells were treated with different concentration of AFB1 ranging from 0.001 to 10 μ M for 24, 48 and 72h. Colony formation was assessed by Crystal Violet staining after 15 days of culture in fresh medium, subsequent to 72h of AFB1 treatment. Apoptosis and cell cycle were assessed after 72h treatment using the Annexin V and Dead Cell Assay kit and the Cell Cycle Assay Kit (Merck Millipore, Darmstadt, Germany), respectively.

Results / Discussion / Conclusion

SRB assay showed a marked reduction of viable cells after AFB1 treatment for all cell lines, with a surprisingly strong effect on MDA-MB-468 cells, as demonstrated by IC₅₀ values (0.62 μ M, 0.45 μ M and 0.044 μ M for MCF7, SKBR3 and MDA-MB-468 cells respectively). To assess if the decreased proliferation was a long-term effect, cells were treated with AFB1 and subsequently harvested and plated at low density for the clonogenic assay; to verify if AFB1 effects was dose-dependent, we carried out the same experiment at two different concentration, IC₅₀ and a lower concentration. After 15 days of culture in fresh medium no colonies were formed in the IC₅₀ treated cells compared to untreated controls, meanwhile the intermediate concentration allowed the formation of few colonies.

Apoptosis and cell cycle analysis were performed: i) in all cell lines, AFB1 did not induce apoptosis activation; ii) MCF-7 and SKBR3 cells did not significantly change the phase distribution after treatment, in accordance to literature [3]; meanwhile iii) MDA-MB-468 cells showed an accumulation in the G2/M phase.

The preliminary results of our study demonstrate a strong inhibiting effect of AFB1 on MDA-MB-468 triple negative cells. This result is interesting because the triple negative subtype represents an ongoing therapeutic challenge.

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Efecto de los métodos domésticos de cocción en firigüero (*Vigna unguiculata*) y zarandaja (*Lablab purpureus*)

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Introducción

Las legumbres corresponden a las semillas secas de las leguminosas que forman parte de la familia de las Fabaceae y tienen origen Africano (Menssen et al., 2017). Las legumbres son una fuente valiosa de nutrientes, como: proteínas, carbohidratos como el almidón y compuestos con una actividad biológica como la fibra dietética, oligosacáridos, minerales, vitaminas, ácidos grasos poliinsaturados, flavonoides y lignanos. Constituyen un alimento clave de la dieta Mediterránea y se las asocia como uno de los factores dietéticos que pueden ofrecer protección contra enfermedades cardiovasculares y el cáncer (Papandreou et al., 2018).

Los métodos de cocción a los que se someten normalmente las legumbres para su consumo, incluyen el hervido, vapor, asado y presión; los cuales inducen una serie de cambios en las características físicas, composición química y modificaciones enzimáticas (Siah, Wood, Agboola, Konczak, & Blanchard, 2014; Tian et al., 2016), razón por la cual el presente trabajo tiene como objetivo estudiar el efecto de los métodos de cocción por ebullición y presión, sobre las propiedades tecnofuncionales, composición nutricional y mineral de zarandaja y firigüero.

Métodos

Se trabajó con firigüero (*Vigna unguiculata*) y zarandaja (*Lablab purpureus*) procedentes de los cantones de Macará y Zapotillo de la provincial de Loja-Ecuador. Las muestras cocidas fueron previamente remojadas durante 5 horas en agua potable, luego sometidas a cocción por ebullición (90°C, 110 – 135 minutos) y presión (120°C, 14 – 25 minutos). Una vez cocidas fueron trituradas y liofilizadas.

Tanto en muestras crudas como cocidas se evaluaron propiedades fisicoquímicas (densidad aparente, pH y acidez), de hidratación (absorción, retención, hinchamiento), de la materia grasa (absorción y retención) e interfaciales (emulsión, espuma, gelificación). En la composición nutricional se evaluó el contenido de agua (AOAC 925.10), cenizas (AOAC 923.03), proteína (AOAC 960.52), grasa (AOAC 920.39), minerales: sodio, potasio, magnesio, calcio, hierro, cobre y zinc por el método AOAC 985.35 y el fósforo por el método AOAC 986.24, AOAC.

Resultados / Discusión / Conclusión

En lo referente a las propiedades tecnofuncionales, las legumbres crudas presentaron un pH entre 7,05 y 7,09 el cual incrementó con la cocción, mientras que la densidad y acidez total disminuyeron. La capacidad de absorción y retención tanto de agua como de grasa se incrementó con la cocción, pero la capacidad de hinchamiento no se vio influenciada. Las dos legumbres poseen alta capacidad para formar espuma (100%) y geles a la concentración mínima evaluada (4%).

La composición nutricional en orden decreciente, correspondió a: carbohidratos (67–71 g/100 g), proteína (21– 29g/100g), agua (11 g/100g), cenizas (2– 4 g/100g) y grasa (1 – 2 g/100g). El potasio fue el mineral más abundante, seguido del magnesio, calcio, zinc, fósforo, hierro, sodio y cobre. El contenido de cenizas, grasas y carbohidratos disminuyó con los dos métodos de cocción y la ebullición afectó en mayor grado a todos los minerales.

Tanto la zarandaja como el firigüero constituyen un recurso promisorio para su uso en el desarrollo de productos saludables y nutritivos para potenciar su consumo e su industrialización.

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Supercritical fluid extraction of tocotrienols from Annato seeds

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Introduction

Bixa orellana L., commonly known as annato, is a plant native of tropical areas, its seeds possess the compounds with the highest antioxidant activity of vitamin E, which has increased interest in its study. The objective of the present investigation was to recover extracts of these seeds using supercritical fluid technology.

Method

The annato seeds were extracted by supercritical carbon dioxide, the effect of the main operation parameters was studied varying temperature from 35 °C to 60 °C, pressure from 10 to 25 MPa and the flow of CO₂ from 20 to 50 L CO₂/min. The extracts were analyzed for their antioxidant capacity using the DPPH method and the content of tocotrienols present in them by GC-MS.

Results / Discussion / Conclusion

The greatest SC-extraction yield was 3.44% corresponding to the conditions of 47.5 °C, 10 MPa and 20 L/min and the antioxidant activity 357.19 μMET/g ext and 185.95 μM EBHT/g ext. While the greatest yield in relation to the contents of δ-T3 and γ-T3 in extracts was evidenced to conditions of 47.5 °C, 10 MPa and 50 L/min of CO₂ corresponding to 10.73% and 0.8% respectively. There by it proved the effectiveness of using supercritical carbon dioxide in the recovery of solvent-free extracts from achiote seeds with an efficient antioxidant capacity.

Anti-Emerging viruses and anti-HHV activities of Callitrisic Acid Derivatives

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Introduction.

Certain mosquito-borne virus, such as Dengue virus (DENV), Chikungunya virus (CHIKV), Zika virus (ZIKV) are an important public health concern in Latin American countries. CHIKV is a RNA genome arthropod-borne-Alphavirus from the *Togaviridae* family. CHIKV infection results in a DENV and ZIKV flavivirus-like febrile disease. At present there are not licensed vaccines or effective antivirals approved for these diseases caused by CHIKV, DENV and ZIKV. HHV is a genomic DNA virus from the *Herpesviridae* family and causes frequent infections in immunosuppressed patients. The long-term administration of acyclovir (ACV) for therapy against HHV infections can result in the emergence of ACV-resistant strains. It is therefore urgent to develop new anti-herpetic compounds with mechanisms that differ from that of ACV. The simplest phenolic abietane diterpenoid, ferruginol, has recently shown antitumor, antiviral and antileishmanial activity. Callitrisic acid is an aromatic abietane compound which has been little studied biologically (Stadler, 2017). Therefore, nine ferruginol analogues derived from Callitrisic acid, isolated from Moroccan Sandarac resin, were synthesized and evaluated for broad-spectrum antiviral activity in this study.

Method.

The antiviral activity against CHIKV, DENV, ZIKV and acyclovir-resistant HHV-29R of Callitrisic acid derivatives were evaluated by TCID₅₀ assay (Tissue Culture Infective Dose) using the end-point titration technique (EPTT) with Vero E6 cells. Antiviral activity-two-fold dilutions of compounds (50 – 6.25 µg/mL) and viral suspension of ten TCID₅₀/0.1mL were mixed and incubated for 15 minutes at room temperature before they were added to confluent monolayer Vero E6 cells and further incubated at 37°C for 48 h, 72 h or 120 h for CHIKV, HHV-1, ZIKV and DENV-2, respectively. After of incubation at 37°C in a humidified 5%-CO₂ atmosphere, medium was removed and cell monolayers were fixed with a solution of formaldehyde (3.5%) and crystal violet (0.4%).

Results.



The nine callitricic acid derivatives showed activity against CHIKV, DENV and ZIKV and/or HHV but none of the compounds showed broad spectrum activity for both RNA and DNA viruses; however, one compound was active against emerging viruses CHIKV, DENV and ZIKV. The antiviral activity was quantified through a reduction factor R_f defined as ratio of the virus titer in the absence over virus titer in the presence of the tested compound. According to the estimate of Vlietinck et al. (see citation in Betancur-Galvis 2002), a purified natural molecule is considered to have a relevant or moderate antiviral activity when the reduction factor (R_f) of viral titer is $\geq 1 \times 10^3$ or 1×10^2 , respectively. The active callitricic acid derivative against emerging viruses CHIKV, DENV and ZIKV at non-toxic concentration of 25 μ g/ml showed viral reduction factor (R_f) among 1×10^2 and 1×10^1 .

Conclusion.

This is the first report about anti-emerging virus activity of tricyclic abietane diterpenes.

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The anti-cancer properties of *Citrus bergamia* juice

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Cancer represents a real emergence, as it accounts for 18.1 million of new cases and 9.6 million of deceases worldwide. In the treatment of this disease, natural remedies are employed both in prophylaxis as well as co-adjuvant of synthetic drugs. During past decades, polyphenolic-rich extracts have attracted attention as potential chemopreventive agents. *Citrus bergamia* Risso et Poiteau (bergamot) is a typical tree of Calabria region (Italy). Its fruits are mainly used for the extraction of the essential oil from the peel, employed for the preparation of fragrances, as well as in aromatherapy (Navarra et al., 2015; Mannucci et al., 2017). Instead, bergamot juice (BJ) was considered a by-product.

In the last decade, we demonstrated that BJ and the flavonoid-rich extract from bergamot juice (BJe) possess noteworthy antioxidant and anti-inflammatory activities, both *in vitro* and *in vivo* models (Risitano et al. 2014; Ferlazzo, et al. 2015; Currò et al. 2016; Ferlazzo, et al. 2016; Impellizzeri et al. 2015; Impellizzeri et al. 2016; Gugliandolo E et al., 2019). Moreover, BJ and BJe have also shown to reduce the growth rate of diverse cancer cell lines through different molecular mechanisms, depending on cancer types. In an *in vitro* model of human neuroblastoma, BJ arrested cell cycle in G1 phase of SH-SY5Y cell line, without triggering apoptosis nor cytotoxicity, yet hindering both cell adhesion and migration. This peculiarity was due to the ability of BJ to induce impairment of actin filaments and reduce expression of active form of focal adhesion kinase (FAK) (Delle Monache et al., 2013). Conversely, BJ diminished growth rate of human hepatocellular carcinoma cells (HepG2), through the activation of both extrinsic and intrinsic apoptosis that involved NF- κ B, p53 and p21 pathways (Ferlazzo, et al. 2016). Furthermore, the BJ-induced reduction of neuroblastoma cells (SK-N-SH and LAN-1) adhesiveness might be responsible for the slight inhibition of pulmonary metastasis colonization in an *in vivo* model of neuroblastoma spontaneous metastases formation in SCID mice (Navarra, et al. 2014). We further indicated that the pool of flavonoids present in BJ is responsible for its anticancer effects. Indeed, BJe is able to inhibit the growth of HT-29 human colorectal carcinoma (CRC) cells by the induction of apoptosis that can be triggered through different mechanisms, depending on the drug concentration (Visalli, et al. 2014). Lower BJe concentrations suppressed MAPK pathways and modulated some proteins linked to the apoptotic machinery, thus inducing arrest of cell cycle and apoptosis. Instead, higher BJe concentrations raised ROS generation leading to both loss of mitochondrial membrane potential (MMP) and DNA oxidative damage (Visalli, et al. 2014). Finally, we showed that daily intake of BJe reduces spontaneous tumorigenesis in the colon of Pirc rats (F344/NTac-Apc^{am1137}), mutated in *Apc*, the key-gene in CRC, through a mechanism involving, at least in part, its anti-inflammatory and pro-apoptotic



activities. The reduction of both colon microscopic preneoplastic lesions and tumours occurs without interfering with the normal mucosa or displaying apparent signs of toxicity (Navarra M. et al., 2019).

Overall, these experimental data document the anti-tumor activity of BJ and BJe, indicating an alternative strategy that could be exploited to fight cancer.

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Argentatine B Derivatives: Comparative analysis of cytotoxic, cytostatic and genotoxic effects

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Introduction

Approximately 12.7 million new cases are diagnosed with cancer annually, and more than 7,6 million die of the disease in the year 2008. This number is expected to increase to 21 million by 2030 (WHO, 2013). An important aim of cancer research is to find therapeutic compounds having high specificity for cancerous cells/tumor and fewer side effects than the presently used cytostatic/cytotoxic agents (Cragg and Newman 2009). *Parthenium argentatum* Gray (guayule), is a rubber plant common to northern México and Southwest Texas. Different triterpenoids have been isolated from resin in abundant quantities, including the Argentatine B (**1**), a triterpeno type cycloartane.

Method

Argentatine B (**1**) were isolated of the resin, a by-product of the industrial process to obtain natural rubber from *P. argentatum*, purified by conventional procedures, and identified by comparison with physical and spectroscopic constants melting point, ¹H and ¹³C Nuclear Magnetic Resonance (Martínez et al., 1990). The synthesis of argentatine B (**1**) derivatives **2-10** was accomplished according to the previous reported proceedings (Parra-Delgado, et al., 2006; Romero-Benavides et al., 2018). All the compounds were characterized by spectroscopic and analytical tools. The cytotoxic activity of argentatine B derivatives for human cancer RKO cell lines is given as the concentration required to inhibit 50% of cell growth of cancer cell line (IC₅₀). Asynchronously growing RKO cells were exposed to the chosen compounds at concentration of IC₅₀ for 24 h, stained with propidium iodide and analyzed by flow cytometry in order to determine the total population distribution in the different phases (G0/G1, S, and G2/M). The damage in DNA was determinate using the comet assay and micronucleus tests. To evaluated the genotoxic effect of Argentatine

B derivatives the lymphocytes were exposure with derivatives of Argentatine B for 3 and 24 hours, depending on the conditions of the tests, comet assay and CBMN, respectively.

Results / Discussion / Conclusion

Ten argentatine B derivatives were synthesized and studied for their inhibitory effects on cell cycle progression in human colon cancer RKO cells. Furthermore the cytotoxic, cytostatic and genotoxic effects of the derivatives on proliferating human lymphocytes were evaluated using the comet and cytokinesis-block micronucleus assays. The results showed that some derivatives have a pronounced effect on cell cycle distribution, caused an arrest of G1. All ten derivatives revealed the genotoxicity to lymphocytes a dose-dependent manner, that slope is different of zero and with correlation coefficient of the model (R^2), greater than 0.88. The derivative **6** showed significant cytostatic effects in RKO and human normal cell, it did not show cytotoxic effects at the same concentrations, and it is the most lower genotoxic effects in terms of tail length and micronucleus frequency in human lymphocytes. Further studies are clearly necessary to elucidate the actual mechanism of action of this cell cycle arrest by these compounds. At present, studies are ongoing in our laboratories to examine other structural properties of Argentatine B derivatives, which might add additional value to their possible use for anti cancer drug in the future.

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Preparations containing "palan-palan"*Nicotiana glauca* (Graham), Solanaceae

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Introduction

Nicotiana glauca n.v “palán palán”, “gandul”. “palqui”, “tree tobacco” among others, is a medium size bush which can reach 6 meters in height, has a bluish green bark, alternate and whole leaves with hairless texture which are slightly fleshy and measure 12 centimeters long. Its yellow flowers in short panicles are 2 to 4 centimeters long; they have a tubular calyx and the corolla has the shape of a long tube. Its fruit is an ovoid capsule with plenty of seeds and comes from Argentina and Bolivia but has been naturalized in different countries. This plant contains alkaloids such as anabasine, pyridine, nicotine, nor nicotine and also citric, succinic, malic, oxalic acid and sterols such as cholesterol, campesterol, sitosterol and stigmasterol, among other active agents. Despite the oral toxicity of its leaves, they have been used by many cultures, such as the Wichi, for skin rejuvenation, wounds treatment, infected ulcers and also for external parasite treatment. They are applied as dressings together with pork fat on the affected area directly.

Methodology

The obtention of different extracts, decoction and dyeing processes were carried out according to the FA VI Edition (Argentine Pharmacopoeia).

The obtained extracts were solubilized with different excipients selected according to the characteristics of the extracts and the intended purpose.

Discussion – Results - Conclusion

This study makes an assessment regarding the different pharmaceutical forms of dermatological use (pomades, ointments, gels) intended to incorporate extracts coming from the *Nicotiana glauca* for its possible use in the treatment of skin diseases.

This preliminary study is intended to be the first step to validate the traditional use of the “palán -palán”.

Simultaneously, it is also aimed at achieving the determination of the most suitable pharmaceutical form for the possible use of “palán-palán” extracts in the preparation of officinal drugs for dermatological use.

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Oleanolic acid as a dual agonist of PPAR γ/α

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Introduction

Diabetes Mellitus type 2 (DT2) is a metabolic disease characterized by defects in glucose metabolism [1]. These defects are due to faults in the secretion and / or action of insulin [2]. Several molecular targets have been reported for the development of therapeutic strategies. The receptors activated by peroxisome proliferators (PPARs) are the most important [3]. These molecules are responsible for maintaining an energy balance, regulating the expression of genes involved in the metabolism of lipids and carbohydrates. PPAR γ exerts a regulation on the metabolism of glucose, through GLUT-4 and adiponectin. PPAR α regulates genes that are involved in lipid metabolism, such as ACSL and FATP-1 [4]. Oleanolic acid (AO) is a natural triterpene compound that has been shown to have important antidiabetic effects [5] by decreasing the concentration of glucose and triglycerides in the blood. This compound represents a novel alternative in the search for molecules capable of activating the expression of PPAR γ and PPAR α . The aim of this work was to determine the effect of oleanolic acid on the expression of PPAR γ , PPAR α , as well as its regulated genes.

Method

C2C12 cell line was cultured and temporal course of PPAR γ and PPAR α mRNA expression was made. A concentration of 10 μ M of oleaolic acid (AO) was used, with incubation times of 15, 30, 60 and 120 minutes. RT-PCR was performed to evaluate the PPAR γ , PPAR α expression, as well as its regulated genes. Western Blot was performed for PPAR γ , PPAR α , as well as their regulated proteins in myoblates treated with oleanolic acid for 2 h. The translocation of GLUT-4 was analyzed by confocal microscopy (Zen-Sp1 ZEISS laser scanning) at 490 nm. Lipid accumulation in adipocytes was quantified through oil red, by spectrometry at 510 nm.



Results / Discussion / Conclusion

The treatment with OA produced an increase in the expression of PPAR γ , PPAR α , AdipoQ, FATP-1 and ACSL. Treatment with OA did not produce significant changes in GLUT4 expression rate. However, AO produces a significant increase in the translocation of GLUT4 quantified by confocal microscopy. The increase in the expression of PPAR γ could be related to that reported by Hsun-Wei *et al* [6], who observed that in the cell line derived from human kidney (HEK 293) increased the expression of PPAR γ using the reporter gene with luciferase6. On the other hand, the AO treatment produced a significant increase ($p < 0.05$) in the protein expression of PPAR γ , PPAR α , GLUT-4 and FATP-1 evaluated by Western Blot. In addition, the AO did not modify the levels of lipid accumulation in adipocytes evaluated by oil red staining. The AO has important effects on the molecular dynamics of both PPAR γ and PPAR α , as well as the genes regulated by these transcription factors. These effects may be associated with its antidiabetic potential. We suggest continuing with the study of this compound on important markers in the insulin signaling pathway.

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Evaluación química y biológica de la microencapsulación por spray drying de los extractos de *Mimosa caesalpinifolia*

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Introducción

Las especies de la familia Mimosaceae se encuentran distribuidas por todo el mundo, y en gran abundancia en la región Neotropical en el Centro de Brasil, considerado uno de los mayores núcleos de diversidad¹. Dentro de esta familia, *Mimosa caesalpinifolia*, es una especie con propiedades medicinales y rica en compuestos fenólicos. El uso de técnicas de microencapsulación es conocido a las décadas, sin embargo viene ganando espacio en las diversas áreas de la industria farmacéutica, alimentaria y cosmética². El proceso de microencapsulación por spray drying tiende a proteger las sustancias sensibles a la luz, el oxígeno, la temperatura y el tiempo de almacenamiento, como los compuestos fenólicos. Además, este proceso puede impedir interacciones con otros compuestos, promoviendo una mayor estabilidad del producto y, consecuentemente, aumentando su vida útil.

El objetivo de este estudio fue evaluar la actividad biológica y química de los extractos secos por spray drying de *M. caesalpinifolia* empleando el Aerosil® 200.

Método

Se extrayeron hojas *M. caesalpinifolia* procedentes del estado de Minas Gerais (Brasil) por percolación hydroethanol 70%. La selección de las condiciones operativas empleadas en el proceso de secado por spray drying se basó en estudios anteriores desarrollados por el prof. Dr. Geraldo Alves da Silva de la Universidad Federal de Alfenas. La estructura de los compuestos fue elucidada mediante técnicas de Resonancia Magnética Nuclear (RMN) de protón y carbono (tanto monodimensional como bidimensional) y espectrometría de masas. Se utilizó la técnica de espectroscopia de Raman y Microscopía Electrónica de Exploración. Los ensayos de Inducción a Colitis, se realizaron en la Escuela Paulista de Medicina-UNIFESP, supervisión de la Prof. Dr. Ana Paula Ribeiro Paiotti.



Discusión de resultados/Conclusion

El adyuvante Aerosil agregado al extracto contribuyó para el mantenimiento de las características físicas del extracto seco, además de influir positivamente en el rendimiento del proceso de secado (55,02%). Esta estabilidad puede atribuirse a una posible microencapsulación de las partículas por Aerosil. Las microencapsulación presentaron tamaños variados, entre 13,62 y 20,78 μm . En el presente estudio biológico el extracto encapsulado (125 mg) fue capaz de inhibir la inmunoexpresión de la COX-2 después de la inducción de la colitis. Por lo tanto, Aerosil ayudó en la formación de estructuras, facilitando la microestructuración de lo extracto. Nuevos ensayos son necesarios para cuantificar el grado de cristalinidad.

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Evaluación de la actividad antioxidante de extractos de polisacáridos de *Pleurotus djamor*

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Introducción

Actualmente la producción de setas ocupa el segundo lugar a escala mundial y ha superado la producción de *A. bisporus* y se espera que esta tendencia se mantenga debido a la relativa facilidad de cultivo y la poca necesidad de grandes inversiones en equipamiento, además de la alta aceptación de los consumidores por sus cualidades nutritivas (Royse *et al.*, 2017). De las aproximadamente 50 especies válidas taxonómicamente para el género *Pleurotus*, al menos 12 han sido cultivadas, entre las de mayor importancia son *P. ostreatus*, *P. pulmonarius*, *P. eryngii* y *P. djamor* (Salmones y Mata, 2017). En los últimos años se han establecido programas de mejoramiento de cepas de *Pleurotus djamor* con el objetivo principal de obtener material genético con características deseables para su producción comercial como: alta productividad, calidad en los basidiomas cosechados (color, tamaño, peso), ciclos cortos y producción estable bajo diferentes condiciones de cultivo (Gaitán-Hernández y Salmones 2008, Guadarrama *et al.*, 2014, Aguilar *et al.* 2018). Por lo que en el presente trabajo se evaluó el cultivo y la actividad antioxidante de extractos de polisacáridos de cepas parentales e híbridas de *Pleurotus djamor* obtenidas por el apareamiento de neohaplontes compatibles.

Método

Se realizó el cultivo en sustrato paja para obtener los cuerpos fructíferos de una cepa parental de *P. djamor* (CC051) y su híbrida (CC051xCC050) posteriormente a partir de la harina de los cuerpos fructíferos, mediante ultrasonificación, seguida de una precipitación con etanol al 80% se obtuvieron los polisacáridos crudos y se caracterizaron por espectroscopia de infrarrojo (IR) y cromatografía en capa fina (CCF). Asimismo, se evaluó la actividad antioxidante con DPPH. **Espectroscopia infrarroja.** Para la caracterización por espectroscopia de infrarrojo (IR), los extractos crudos de polisacáridos se analizaron en un equipo de espectroscopia infrarrojo Perkin-Elmer FT-IR modelo System Spectrum GX, a una longitud de onda de 550 cm⁻¹ a 4000 cm⁻¹. Los espectros, se analizaron con el software Spekwin32, para identificar grupos funcionales característicos de los polisacáridos. **Cromatografía en capa fina:** Una vez obtenido el extracto crudo, se hidrolizó y se sometió a un proceso de separación en cromatoplasas de aluminio utilizando como fase móvil mezclas de los solventes butanol: propanol: agua (3:12:4) y como revelador mezcla de difenilamina, anilina, ácido fosfórico en etanol absoluto. **Evaluación de la actividad de captación de radicales libres (DPPH):** Para obtener la curva patrón se utilizó ácido ascórbico con una concentración de

0.24 mg/mL. Las muestras se prepararon con una concentración de 40 mg/mL de extracto de polisacáridos, se mantuvieron en obscuridad a 25°C durante 30 minutos. Las absorbancias se midieron a 515 nm en un espectrofotómetro marca Thermo Electron Corporation modelo Genesys 10UV.

Resultados / Discusión / Conclusión

La cepa híbrida obtuvo mayor productividad (35.36% EB) con respecto a la cepa parental (22.66% EB). Se obtuvieron menores rendimientos de los extractos de polisacáridos en la cepa parental (10.48%) en comparación con la cepa híbrida (12.40%). Los espectros IR mostraron grupos funcionales característicos de los polisacáridos de *Pleurotus*. El contenido de azúcares totales fue de 34.76 y 34.32% para la cepa parental e híbrida, respectivamente. La cromatografía en capa fina indicó la presencia de monosacáridos glucosa, manosa y arabinosa en los extractos de polisacáridos. Los valores de la concentración inhibitoria media (IC₅₀) de la actividad antioxidante de las cepas fueron de 29.25 mg/mL para la cepa parental y de 8.83 mg/mL para la cepa híbrida. Xia *et al.* (2011) reportó valores 9.7 mg/mL para extractos crudos de polisacáridos de *P. ostreatus*. Por lo que con los polisacáridos de la cepa híbrida se obtuvo un valor menor de concentración media inhibitoria y representa un extracto potencial como antioxidante. Los resultados obtenidos sugieren que la cepa híbrida representa un genotipo importante para la producción de polisacáridos de interés biológico y para la actividad antioxidante de los mismos.

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Identification of polyphenols in the ethanolic extract of the leaves of the medicinal plant *Urera baccifera* by FIA/ESI/IT/MSⁿ

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Introduction

In Cuba, *Urera baccifera* (L.) Gaudich (belonging to Urticaceae family) is known as **chichicate**. In this Caribbean island it is recommended the infusion of its roots for expelling kidney stones and as a diuretic, while in Brazil and other Latin American countries the population uses its leaves for skin treatment, urinary infections and as anti-inflammatory. Pharmacological studies performed on both the roots and the leaves of this plant have demonstrated moderate antioxidant, genotoxic and antiviral activities. From the chemical point of view, the quantification of total polyphenols, flavonoids, flavonols, condensed tannins, and alkaloids has been reported by different authors, with marked differences in the results depending on the place of collection. However, there is no report about the identification of secondary metabolites in their extracts, so in this work we aim to identify the majority polyphenols present in the ethanolic extract of its leaves by FIA / ESI / IT / MSⁿ.

Method

The plant was collected in Jaruco, Mayabeque in May 2018. Its dried and ground leaves were degreased by decoction with n-hexane for 4 hours, then filtered and extracted again by decoction with ethanol

The ethanolic extract was concentrated to dryness. The chemical composition of the ethanolic crude was determined by the FIA/ESI/IT/MSⁿ technique on a Thermo Finnigan LCQ Deca mass spectrometer (San Jose, CA, USA) equipped with an ESI source and ion trap analyzer. A preliminary analysis was performed in the positive and negative modes, finally choosing the negative mode for the generation and analysis of the spectra. The first and second order spectra were obtained in the range of m/z 50-2000. Data acquisition and processing was performed using the Xcalibur software.

Results / Discussion / Conclusions

The analysis of the second order spectra corresponding to the deprotonated fragment ions (M-H)⁻ of greater relative abundance corresponds to phenolic acids such as quinic acid, chlorogenic acid and others, we also

find the presence of flavonoids with substituents derived from phenolic acids, such as, dihydrocaffeic and ferulic acids bonded to apigenin, also caffeic and coumaric acids bonded to kamferol. In the work the corresponding fragmentation that justifies the presence of these metabolites in the ethanolic extract of the leaves of *Urera baccifera* that grows in Jaruco, Cuba, is exposed.

Efecto protector del extracto orgánico *Cnidoscopus chayamansa* contra daño hepático inducido con fármacos antituberculosos

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Introducción

Existen más de 1100 fármacos de uso actual que causan hepatotoxicidad, entre ellos están los fármacos antituberculosos (anti-TB) como Rifampicina (RIF), Isoniazida (INH) y pirazinamida (PZA). El daño hepático es uno de los principales problemas en el tratamiento de la tuberculosis (TB) por su duración, esto ocasiona abandonan el tratamiento y favorece la aparición de casos MFR y XFR.^{1,2} Por ello, es necesario contribuir en la búsqueda de sustancias o compuestos que ayuden a prevenir el daño hepático causado por los medicamentos anti-TB. En un estudio previo se demostró que el extracto etanólico de *Cnidoscopus chayamansa* protege del daño hepático inducido por la mezcla de RIF e INH en ratas Wistar, al ser administrado por 21 días.³ En el presente trabajo se reporta el efecto hepatoprotector del extracto de las hojas de *C. chayamansa* contra hepatotoxicidad inducida por la mezcla de fármacos anti-TB (RIF, INH y PZA) durante 40 días⁴.

Método

Material Vegetal y obtención de extractos: El extracto CHCl₃:MeOH de *C. chayamansa* (CNII) se obtuvo por maceración de las partes aéreas a temperatura ambiente, obteniendo un rendimiento del 9.9%.⁵ El daño hepático se indujo con la mezcla de antiTB: RIF/INH/PZA (50/50/100 mg/kg), administrado por vía oral durante 40 días, el extracto y silimarina se solubilizaron en carboximetilcelulosa al 0.5% (CMC). Los grupos de experimentación fueron: grupo I: control; grupo II: Fármacos anti-TB + Vehículo; grupo III: Fármacos anti-TB + Silimarina (2.5 mg/kg); grupo IV: Fármacos anti-TB + CNII (200 mg/kg) y grupo V: Fármacos anti-TB + CNII (400 mg/kg). Al término del tratamiento, se obtuvo sangre para análisis de química sanguínea y estrés oxidativo. Por otro lado, se extrajo el hígado para análisis histológico.

Resultados / Discusión / Conclusión

El extracto CNII a la dosis de 200 mg/kg favoreció la ganancia de peso corporal (GPC), siendo esta ganancia que el de animales sanos y silimarina. El grupo II (daño hepático) mostró menor GPC. Por otro lado, en el perfil hepático, se observó que el extracto CNII a 200 mg/kg provocó disminución en los niveles de AST, ALT y ALP respecto al grupo con antiTB y al control positivo (silimarina), siendo mejor esta dosis que la de

400 mg/kg. Los marcadores de estrés oxidativo mostraron un incremento de lípidos oxidados en el grupo de fármacos anti-TB, valores que disminuyeron en los grupos de silimarina y los extracto a ambas dosis, mostrando mejor respuesta el grupo de silimarina (disminución del 28.65%), mientras que el CNII a 200 y 400 mg/kg provocó una disminución del 22.47 y 35.95%, respectivamente. El análisis histológico del grupo de antiTB indicó presencia de esteatosis, efecto que fue menor en los grupos de silimarina y extracto CNII a 200 y 400 mg/kg. En conclusión el extracto CNII mostró efecto hepatoprotector, debido a que favoreció la ganancia de peso, disminuyó los niveles de AST, ALT ALP, LPx y disminuyó la esteatosis, respecto al grupo antiTB.

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Actividad antibacteriana de 10 especies de plantas de la región Caribe colombiana contra *Staphylococcus aureus* sensible y resistente a meticilina

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Introducción

La resistencia bacteriana a los antibióticos de uso clínico es un problema que amenaza la salud mundial, presentando un aumento en las tasas de morbilidad y mortalidad, costos de la atención médica y disminución en la calidad de la atención¹. Debido a la eficacia de las plantas para contrarrestar las infecciones bacterianas y la abundancia de moléculas con diferentes estructuras químicas (metabolitos secundarios) en estas, existe un creciente interés en el potencial de los recursos vegetales no explotados, para encontrar nuevas moléculas y farmacóforos para el desarrollo de una nueva generación de fármacos antibacterianos². En este estudio se evaluó la actividad antibacteriana de extractos etanólicos de diez especies vegetales *Ludwigia helminthorrhiza* (Mart.); *Ludwigia erecta* (L.); *Ludwigia leptocarpa* (Nutt.) H.Hara; *Sagittaria lancifolia* (L.); *Mammea americana* (L.); *Echinodorus paniculatus* (M.); *Maclura tinctoria* (L.) Steud., *Eleocharis elegans* (Kunth) Roem. & Schult., *Echinodorus tunicatus* Small y *Dicliptera sexangularis* (L.) Juss, contra cepas de *Staphylococcus aureus* sensible y resistente a meticilina.

Método

Las especies vegetales fueron recolectadas en diferentes municipios del departamento de Bolívar, al norte de Colombia. Se prepararon 20 extractos etanólicos a partir del material vegetal de las 10 especies. Se evaluó la susceptibilidad de dos cepas de *Staphylococcus aureus*, ATCC 29213 (sensible a meticilina) y ATCC 43300 (resistente a meticilina) a los extractos etanólicos por el método de microdilución en caldo³. Los extractos fueron disueltos en dimetilsulfoxido, diluidos en caldo Mueller Hinton ajustado con cationes y evaluados a una concentración final de 512 µg/mL (50 µg/mL para el extracto de semillas de *Mammea americana*). El inóculo bacteriano usado fue de 1x10⁵ UFC/mL. Como control positivo de actividad antibacteriana se utilizó una solución de gentamicina de 10 µg/mL. Como control negativo se usó una concentración de

dimetilsulfoxido a 1,25%. Se calculó el porcentaje de inhibición del crecimiento basado en la medición de formazan a partir del reactivo *p*-iodonitrotetrazolium.

Resultados / Discusión / Conclusión

Los extractos de las especies *Mammea americana* (L.)-Raíz, *Ludwigia helminthorrhiza* (Mart.), *Ludwigia leptocarpa* (Nutt.)-Hojas y *Ludwigia erecta* (L.) H.Hara-Hojas, presentaron porcentajes de inhibición mayores del 70% contra las dos cepas de *S. aureus*, por lo tanto, se consideraron promisorios para el fraccionamiento biodirigido y aislamiento de compuestos a partir de la fracción más activa.

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Plants used for cosmetic purposes

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Introduction

Preparations based on medicinal plants (MP) are commonly used for cosmetic purposes and skin and hair care. The aim of this study was to describe the use of some cosmetic preparations for skin and hair care based on natural products derived from medicinal plants.

Method

Relevant studies of MP were systemically retrieved from electronic databases from PubMed, EMBASE, and textbooks were employed for analyses. The information regards to antipathogenic substances and medicinal properties of MP related to skin and hair conditions were compiled. Besides, the utilization of MP and its derivatives in skin and hair disorders was gathered. Finally, the cosmetic uses of MP were studied.

Results

We described the main pharmacological and therapeutic properties of MP related to the skin and hair care, as well as, with conditions of these organs. Besides, the main pathologic conditions of skin and hair that could be treated with products based on MP are described.

Discussion and Conclusion

Products contained in plants used in phytotherapy have mainly free-radical scavenging, anti-inflammatory, astringent, emollient, properties. These characteristics elicited the formulation of a series of products for skin and hair products aimed to improve acne, dryness, skin ageing, and other skin conditions. Essential oils incorporated in products provided therapeutic effects and a pleasant aroma. Besides, products for hair care are commonly used as colorants and in dandruff disorder.

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Comparative study of the chemical composition and antioxidant activity of different extracts of *Ilex paraguariensis*

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Introduction

The use of plants for medical treatments occurs since remote times. *Ilex paraguariensis* may be a promising alternative for many diseases. It is native to South America, used in popular medicine as well as raw material for beverages, such as *chimarrão*, *terere* and mate tea. Their extracts have a great antioxidant capacity¹. However, the composition can be influenced by production processes such as drying and roasting, including the extraction itself. This work proposes a comparison of the chemical composition and the antioxidant activity of three different forms of *I. paraguariensis* consumption: *chimarrão*, *terere* and mate tea.

Method

The three different extracts were prepared from a commercial brand of *I. paraguariensis*. The extraction procedure of each was performed in triplicate, mimetizing the popular preparation,^{2,3} and lyophilized for analysis. The composition analysis was performed using a HPLC reverse phase technique, based on Colpo et al². The quantification of 5 components was made: Caffeine, theobromine, caffeic acid and chlorogenic acid and gallic acid. The chromatographic conditions of operation were: Column C-18 protected by a C-18 column guard. A linear gradient elution mode was used, with a flow rate of 1.0 ml/min and an injection volume of 20 µL, using a mobile phase consisting of (A) water with 0.3% acetic acid and (B) methanol following the gradient profile: 15-20% B per 20 min; 20-85% B for 5 min; 85% B for 10 min. The detection was monitored at 265 nm for caffeine and theobromine and at 325 nm for caffeic chlorogenic and gallic acids by DAD system. The samples were analyzed for their total soluble phenol content using the Folin-Ciocalteu⁴ method and the results expressed as g of gallic acid equivalent (GAE) per 100 grams of dry basis. For the analysis of the antioxidant activity, were performed ORAC⁵ and DPPH⁶ tests and the results expressed as µmol Trolox equivalent/g extract and an estimate of IC50 (µg/mL) respectively. The results were analyzed by one way ANOVA. Differences were considered significant when p<0.05.

Results / Discussion / Conclusion

In the composition analysis performed by HPLC, all extracts presented chlorogenic acid as a major element (27 - 42mg/g), followed by caffeic acid (23 - 26mg/g), caffeine (13- 21mg/g) and theobromine (2 - 6mg/g),

except for mate tea, where the concentration of caffeine (~19mg/g) was higher than caffeic acid (~13mg/g). These results agree with data previously described in literature⁷. A comparison between the three preparations showed that mate tea extracts had significantly lower concentrations than *chimarrão* of all analyzed components. Regarding the content of total soluble phenolics, *chimarrão* extract showed higher concentration (~16g GAE/100g), followed by mate tea and *tereré*. In both tests related to antioxidant activity, *chimarrão* presented the most promising results, with a ORAC value approximate 9000 μ mol Trolox equivalent / g extract and an IC₅₀ of 14 μ g/mL with DPPH test. In fact, the antioxidant activity of *I. paraguariensis* extracts is mainly related to the content of phenolic compounds⁷. In addition, Xu and Chang⁸ correlated the content of chlorogenic acid and caffeic acid with this property.

Our results indicate a difference in the composition of the three extracts prepared from a mimetic consumption manner. In this context, *chimarrão* was the one that presented greater concentration of total soluble phenolics, higher concentration of the standards analyzed components, and more promising antioxidant activity.

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Cumarines from extracts of aerial parts of *Chiliotrichum diffusum* (Asteraceae), a medicinal plant used by the onas

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Introduction

Chiliotrichum diffusum, “mata negra”, “kóor” (in Shélnam or Ona language), a medicinal plant used in traditional medicine of the Onas (original peoples of Patagonia), inhabits the southwest of Argentina.

Flowers and leaves have been used to treat cramps, varices, pain and improve memory; flowers have been used to “clarify the sight”. In previous studies we have researched flavonoids, phenolic acids and terpenoids, which are related to the traditional uses and bioactivity analyzed (antioxidant, antitumor, cytotoxic, analgesic, anti-inflammatory, hypotensive and gastroprotective activity)^{1,2}.

In this work, we analysed in the aerial parts the presence of coumarins, in order to increase the knowledge of the metabolites related to the pharmacological activities studied and its traditional use¹.

Methods

Aerial parts of *Chiliotrichum diffusum* (G. Forst.) Kuntze (Asteraceae) were collected in 28 de Noviembre city (Santa Cruz, Argentina). Air-dried and powdered aerial parts were extracted with ethanol or by decoction^{3,4}.

Coumarins were studied by TLC on Silica gel G60 F₂₅₄. We used two systems, system 1: toluene-ethyl ether (1:1 / saturated with acetic acid 10 %) and system 2: ethyl acetate-glacial acetic acid-formic acid-water (100:11:11:26). Both systems were analyzed to UV 365 and 254 nm without and with chemical treatment⁵.

Results / Discussion / Conclusion

Chromatographic profiles showed coumarins in both extracts of *C. diffusum*. System 1 showed scopoletin and umbelliferone in both extracts, while isofraxidin and xanthotoxin were only found in the ethanol extract, accompanied by caffeic acid. System 2 showed coumarin aglycones, mainly umbelliferone and scopoletin in both extracts, although more prominent in the ethanol extract, accompanied by phenolic acids, rutin, quercitrin and flavonoid glycosides.



Coumarins are a group of natural metabolites derived from benzopyrone, which have an important role as chemical defenses. These have biological activities such as antioxidant, analgesic, anti-inflammatory, antitumoral and antimicrobial. They stand out among other families, in the Asteraceae; particularly umbelliferone, scopoletin and herniarin in *Matricaria chamomilla* (chamomile)⁶. Ours results show that, as with other metabolites, *C. diffusum* presents a metabolic profile similar to chamomile, a plant drug widely used medicinally. At the same time, the coumarins detected, added to the flavonoids and phenolic acids, are related to the traditional medicinal uses and to the biological activities determined previously, fundamentally as antioxidant, antimicrobial and anti-inflammatory.

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Evaluación de la Actividad Antioxidante en Plantas de la familia Melastomataceae

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Introducción

El estrés oxidativo se ha involucrado en la fisiopatología de diversas enfermedades como las neurodegenerativas, aterosclerosis, cáncer, así como en el proceso normal de envejecimiento (Leos-Rivas et al., 2016). La evaluación de la actividad biológica de extractos obtenidos a partir de diversas especies vegetales ha sido importante en la búsqueda de moléculas con actividad antioxidante que puedan emplearse como alternativas para el control del estrés oxidativo (Li et al., 2014). Algunas especies de plantas de la familia Melastomataceae se emplean en la medicina tradicional para la elaboración de fitofármacos (Jiménez et al., 2015). Teniendo en cuenta lo anterior, el presente estudio tiene como objetivo evaluar la actividad antioxidante de extractos de plantas de la familia Melastomataceae, que conforman la biodiversidad colombiana.

Método

Actividad antioxidante por el método DPPH. 25 μ L de los extractos y Trolox, a diferentes concentraciones en metanol, y los respectivos controles, se agregaron a una placa transparente de 96 pozos seguidos de 100 μ L de una solución de DPPH a 20 μ g/mL. La placa con la mezcla se incubó en oscuridad a temperatura ambiente durante 30 minutos, y finalmente se midió la absorbancia a 517 nm. Se determinó el valor IC₅₀ en μ g/mL, por medio de una regresión no-lineal empleando el programa GraphPad Prism 6.

Actividad antioxidante por el método ORAC. 30 μ L de los extractos y Quercetina, a diferentes concentraciones en solución buffer fosfato 75 mmol/L, y los respectivos controles, se adicionaron a una placa de 96 pozos seguidos de 180 μ L de una solución de Fluoresceína a 120 nmol/L. La mezcla se incubó en oscuridad a 37°C durante 15 minutos, se adicionaron 30 μ L de solución AAPH a 140 mmol/L y se procedió con la lectura de la fluorescencia con λ de excitación de 485 nm y λ de emisión de 538 nm, durante

120 minutos en intervalos de 1 minuto. La capacidad antioxidante se calculó como el área bajo la curva y se expresó como μmol equivalentes de Trolox/g de extracto ($\mu\text{mol ET/g}$).

Resultados / Discusión / Conclusión

Se evaluaron 13 extractos de siete especies de plantas de la familia Melastomataceae. Los extractos de *Tibouchina ciliaris*-Acetona 70%, *Miconia caudata*-acetato de etilo y *Miconia coronata*-acetato de etilo y butanol, mostraron los siguientes valores 16254 ± 5092 , 20847 ± 2313 , 19503 ± 2058 , 15967 ± 1688 $\mu\text{mol ET/g}$, respectivamente por el método ORAC, respecto a Quercetina (22084 ± 2928). Por otro lado, la actividad antioxidante por DPPH de *Tibouchina ciliaris*-Act 70% (5.13 ± 0.68 $\mu\text{g/mL}$) y *Miconia prasina*-AcOEt (6.93 ± 0.86 $\mu\text{g/mL}$), fue superior a la del estándar Trolox (12.24 ± 0.26 $\mu\text{g/mL}$), mientras que *M. caudata*-AcOEt (15.58 ± 2.20 $\mu\text{g/mL}$), *M. coronata*-AcOEt y BuOH (14.17 ± 1.21 y 14.28 ± 1.22 $\mu\text{g/mL}$), *Clidemia hirta*-AcOEt (9.36 ± 0.90 $\mu\text{g/mL}$), *Miconia sp.*-Acuoso y BuOH (9.12 ± 0.42 y 9.67 ± 0.97 $\mu\text{g/mL}$) y *Miconia prasina*-Act. 70% (10.11 ± 2.10 $\mu\text{g/mL}$) presentaron actividad similar a Trolox. Los extractos que presentaron actividad antioxidante significativa con respecto a los estándares, son una fuente de compuestos para el control del estrés oxidativo, teniendo en cuenta que la identificación preliminar de los núcleos fitoquímicos ha mostrado la presencia de compuestos fenólicos, flavonoides y taninos, a los que se puede atribuir la actividad evaluada.

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Evaluación de diversos compuestos como agentes moduladores del quorum sensing en la especie de *Microcystis aeruginosa*

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Resumen

Microcystis aeruginosa es una especie de cianobacteria que se encuentra presente en diversos ecosistemas; su crecimiento está relacionado con diversos factores ambientales, como la temperatura, el contenido de nitrógeno (N) y fósforo (P), pH, intensidad de luz, etc.[1]. En Antioquia, embalses como Porce y Riogrande han dado reportes de presencia de esta especie. La presencia de estos microorganismos en los cuerpos de agua representa un riesgo, debido a que es una especie productora de cianotoxinas, llamadas microcistinas (MCs); La de mayor impacto debido a su toxicidad es MC-LR, la cual es un heptapéptido que causa severos daños en el organismo e incluso la muerte.[2]

El crecimiento y la biosíntesis de toxinas puede ser regulado por factores exógenos.[3] la especie *Microcystis aeruginosa*, no es ajena a estos procesos, se han reportado interacciones del tipo planta microorganismo en las cuales moléculas producidas por plantas son capaces de regular el crecimiento de esta cianobacteria[4], sin embargo, el efecto de algunas moléculas que son arrastradas hasta los embalses como medicamentos no ha sido estudiado. Por tanto, el objetivo de este estudio fue evaluar el efecto de varios antibióticos usados a nivel ganadero y moléculas aislados de comino crespo sobre el crecimiento y la biosíntesis de toxina.

Los cultivos de *Microcystis Aeruginosa* se incuban a temperatura ambiente, iluminación, por lámparas de luz blanca (bajo condiciones de ciclo de luz/oscuridad de 12h/12h) y aireación constante. Las concentraciones evaluadas de los antibióticos son de 0,01µM, 0,1µM, 1µM y 10 µM rango en el cual se ha reportado capacidad moduladora de crecimiento por un lapso de 78h. Se realiza un conteo de células y colonias y se cuantifica MC-LR a través de cromatografía líquida de alta resolución. La mejor respuesta en cuanto al crecimiento y la producción de toxina se encontró después de la aplicación de Gentamicina, Penicilina y Tetraciclina, confirmando que los medicamentos que llegan por las malas prácticas agrícolas inciden en la proliferación de cianobacterias.

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Actividad antiinflamatoria de *Cleoserrata serrata* (Jacq.) Iltis.

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Introducción

Cleoserrata serrata (Jacq.) Iltis. (Syn. *Cleome serrata* Jacq.) es una herbácea ruderal de la familia *Cleomaceae*, con escasos estudios químicos y biológicos. El género se distribuye al sur de México, América Central, América del Sur y las Antillas. Es conocida como “mastuerzo” o “zumkak” y es empleada en la medicina tradicional del estado de Tabasco, México para el tratamiento de lesiones cutáneas conocida como “úlceras del chiclero” (provocado por *Leishmania*). El extracto CH₂Cl₂:MeOH (1:1) de las hojas de *C. serrata* fue activo contra amastigotes y promastigotes de *Leishmania mexicana* con una DL₅₀ = 6.11 y 23.5 µg/mL, respectivamente; además, mostró moderada actividad contra *Staphylococcus aureus*, *Escherichia coli* y *Pseudomonas aeruginosa*.^{1,2} En este trabajo se reporta el potencial antiinflamatorio del extracto CH₂Cl₂:EtOH y de las fracciones primarias de *C. serrata* (partes aéreas secas) en dos modelos de inflamación *in vivo* tópico y sistémico, así como su perfil fitoquímico.

Método

C. Serrata se colectó en Coatepec, Veracruz, México, y un ejemplar (voucher 16,307) se depositó en el Herbario FESZ, UNAM. Las partes aéreas secas y molidas (550 g) se maceraron con CH₂Cl₂:EtOH (1:1) para obtener el extracto (198.93 g) cuyo rendimiento fue de 36 %; el cuál fue fraccionado por cromatografía en columna abierta y se eluyó con Hexano, AcOEt, EtOH y mezclas de estos. Mediante el fraccionamiento se obtuvieron 107 fracciones que fueron agrupadas (por su perfil cromatográfico) en 21 grupos. Las fracciones poco polares contiene ácidos grasos, esteroides y triterpenos, estos fueron detectados por Cromatografía de Gases acoplado a Espectrometría de Masas (CG-EM) y las fracciones polares contienen polifenoles, estos metabolitos fueron detectados por ccf. La actividad antiinflamatoria aguda sistémica y tópica del extracto y fracciones primarias se determinó en ratones macho Balb/c, empleando el modelo de TPA y carragenina e indometacina como referencia.

Resultados / Discusión / Conclusión

El análisis por CG-EM y ccf permitió determinar que las fracciones contienen como compuestos mayoritarios: fitol, β sitosterol, ácido palmítico, γ-sitosterol o clionasterol y estigmastan-3,5-dieno. En las fracciones polares se detectó al menos 4 polifenoles mayoritarios con núcleo base de kaempferol.

El extracto CH₂Cl₂:EtOH (1:1) a 300 mg/kg inhibió el 40% la formación del edema a la 5ta h, y la indometacina inhibió el 51%. Las fracciones primarias evaluadas a 150 mg/kg (administrada por vía i.g) mostraron los siguientes % de inhibición: F47-54 (40%), F62-68 (51%), F85-90 (41%) y F104-107 (59%), resultando más activas que indometacina.

Por el modelo TPA, del extracto CH₂Cl₂:EtOH a 2 mg/oreja inhibió el 63% de la inflamación y las fracciones más activas que el fármaco de referencia (indometacina, 44 % de inhibición) fueron: F62-68 (75.43%), F15-16 (71.92%), F101-103 (50.87%), F47-54 (49.12%) y F85-90 (47.95 %). Estos resultados evidencian por primera vez *C. serrata* es una fuente potencial de compuestos antiinflamatorios

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The effect of multiple polarities *Tecoma stans* extracts on lipid profile in mice fed a hypercholesterolemic diet

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Introduction

One of the most prevalent diseases in the Mexican population is hyperlipidemia consequently causing cardiovascular diseases, among others. Hyperlipidemia is the increase in the amount of lipids above established levels and hypercholesterolemia is the increase in the amount of cholesterol above set standards. Due to these major complications, it is necessary to seek adequate treatment to control these conditions. Nowadays, the existing treatments are insufficient since, in many cases, these treatments are chronic and prone to generate adverse events. For instance, statins, which inhibit cholesterol synthesis, produce liver damage in patients who have used them chronically. Therefore, it is necessary to explore new safe and harmless therapeutic alternatives capable of normalizing lipid and serum glucose levels. Based on the activity reported by the Mexican population on the use of the *Tecoma* plant, in the present investigation, the hypolipidemic activity of the extracts of different polarity of this plant was evaluated.

Method

Male ICR mice (28-30 g weight) were used, acclimatized for 7 days with 12 light/dark hours, 22°C, with water and food ad libitum; the animals were randomly distributed in 11 groups (n=8), 1) normal diet (DN), 2) hypercholesterolemic diet (DH), 3) hypercholesterolemic diet + infusion 25% (DH+ WE25), 4) hypercholesterolemic diet + infusion 50% (DH+ WE50), 5) hypercholesterolemic diet + 100% infusion (DH+ WE100), 6) hypercholesterolemic diet + 100 mg/kg methanolic extract (DH+ ME100), 7) hypercholesterolemic diet + 200 mg/kg methanolic extract (DH+ ME200), 8) hypercholesterolemic diet + 300 mg/kg methanolic extract (DH+ ME300), 9) Hypercholesterolemic diet + 100 mg/kg ethyl acetate extract (DH+ AE100), 10) Hypercholesterolemic diet + 200 mg/kg ethyl acetate extract (DH+ AE200) and 11) Hypercholesterolemic diet + 300 mg/kg ethyl acetate extract (DH+ AE300) and treatment was administered intragastrically daily for 7 days. At the end of the study, blood samples were collected and centrifuged to obtain serum, the mice were sacrificed following the Laboratory Animal Guidelines of NOM-ZOO-062-1996. Total cholesterol, triglycerides and HDL-C were determined by means of RANDOX kits in a autosampler Wiener Lab Selectra.

Results / Discussion / Conclusion

The administration of the *Tecoma stans* infusion showed no decrease in cholesterol, HDL or triglyceride levels, perhaps because compounds with activity are not soluble in polar solvents such as water. The results of the administration of the methanolic extract followed the same trend as the treatment with the first extract: no significant differences in the change of the quantified lipid levels were obtained. While, with the administration of ethyl acetate extract, it was observed that the percentage of cholesterol decrease comparing the group with the hypercholesterolemic diet and the group treated with the 200mg/kg dose was 24.75%. Based on this, it is concluded that the ethyl acetate extract of *Tecoma stans* presents a hypolipidemic activity at a dose of 200 mg/kg. This result suggests that the active metabolites are of low polarity. It has been described in other studies that this fraction separates alkaloids and flavonoids, phenolic acids, amongst others. Likewise, the pharmacological activity of these compounds has been reported in other plants, which suggests that in the current study is due to similar compounds.

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Evaluación de la actividad antioxidante de *Senecio cinerarioides* colectado en el municipio de Chilapa, México

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Introducción

Las plantas poseen una gran cantidad de metabolitos primarios y secundarios que le permiten crecer, multiplicarse, defenderse y sobrevivir. El metabolismo secundario de las plantas puede definirse como el nivel funcional del metabolismo que es para la supervivencia de la especie (1). La planta *Senecio cinerarioides* es muy común en municipios de la parte alta del Estado de Guerrero como también en el Valle de México existe en abundancia(2) se extiende en parte de los Estados como Tlaxcala y Morelos. Se cuenta únicamente con información de conocimiento empírico acerca del uso de la planta, como efecto relajante específicamente en su olor, cuando las personas presentan migraña o dolor de cabeza.

Método

Las hojas y flor fueron colectadas del municipio de Chilapa, Gro, México. Se dejaron secar durante una semana, después fueron trituradas y depositadas en recipientes de vidrio con etanol para la maceración en frío y extraer el extracto total. Para fraccionar el extracto total se utilizaron solventes de polaridad ascendente: hexano, diclorometano y acetato de etilo y se evaporaron en un rotavapor. Las determinaciones fueron por triplicado y empleando espectrofotometría. Se determinaron el contenido de Polifenoles Totales mediante el método de Folin-Ciocalteu(3), Flavonoides Totales mediante el ensayo de cloruro de aluminio(4) y la Actividad de eliminación de radicales libres DPPH se evaluó usando 2-2-difenil-1-picrilhidrazilo⁵.

Resultados / Discusión / Conclusión

De los extractos obtenidos a partir de hoja y flor de *Senecio cinerarioides*, para la cuantificación de polifenoles totales se utilizó ácido gálico, para flavonoides totales se utilizó quercitina, así como también la evaluación de la actividad antioxidante utilizando el reactivo TROLOX como control positivo (52µg/ml) demuestra que el acetato de etilo en flor (64 µg/ml) es el disolvente con mayor capacidad de reducir el 50% de concentración inhibitoria.



Flavonoids are the most widely distributed group of phenolic compounds, normally they are attributed for the antioxidant properties. In this study, the total flavonoid content of this extracts and their fractions was evaluated by the colorimetric assay of AlCl_3 . Quercetin was used as a standard, and total flavonoid content is expressed in mg Quercetin Equivalent per gram of extract (QE mg/g). The results are shown in Table 1. Similar to what was observed in the total phenols content, EtOH extract of *H. brasiletto* showed the highest total flavonoids content (23.5 QE mg/g), however in this case the EtAc fraction of *F. obtusifolia* (23.0 QE mg/g) does not showed significant difference in the total flavonoids content in comparison to the EtOH extract of *H. brasiletto*, which means that the total phenols content in both samples are not flavonoid type

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Potencial de cultivo probiótico de *Lactobacillus acidophilus* en maíces nativos antioxidantes

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Introducción

Los alimentos funcionales con probióticos y/o antioxidantes polifenólicos han demostrado diversos efectos benéficos a la salud tanto preventivos como correctivos, por ejemplo; actividades antiinflamatoria, anticancerígena, antiapoptótica, así como el abatimiento del estrés oxidativo y algunos casos de diarrea. México es el centro de origen y domesticación del maíz (*Zea mays*), de las más de 220 razas registradas al menos 65 tienen presencia en el país, los maíces nativos contienen pigmentos polifenólicos antocianínicos con potencial antioxidante para la formulación de alimentos funcionales sinérgicos en combinación con microorganismos probióticos. En el presente trabajo se realizaron ensayos para testar la adaptabilidad de *Lactobacillus acidophilus* para el desarrollo de bebidas probióticas a partir de maíces nativos antioxidantes.

Método

Se colectaron 8 variedades de maíces nativos del estado del Norte de Jalisco, México, los cuales fueron identificados a nivel de raza de acuerdo a la CONABIO. Se realizó una molienda de los maíces a un tamaño de partícula de 149 µm y posteriormente se realizó una extracción metanólica de fenoles totales que fueron cuantificados por el método de Folin-Ciocalteu usando ácido gálico como estándar. A su vez, se caracterizó la actividad antioxidante de los extractos por la extinción del radical DPPH empleando Trolox (Sigma) como referencia. La cepa ATCC 4356, *Lactobacillus acidophilus* fue cultivada en ausencia y presencia de 100 g/L de maíz rojo güino para: medio MRS (MERCK), leche entera y buffer acetatos de sodio 100 mM, pH 4.5 adicionado de 5g/L. Se usó maíz blanco híbrido de variedad local como control. Se determinó a los cultivos consumo de azúcares por la técnica de DNS y se midió acidez titulable con una solución de Hidróxido de sodio para la cuantificación de ácido láctico. Los cultivos fueron realizados en anaerobiosis a 15 cmHg de presión negativa por 72 horas y 40 °C. Todos los ensayos analíticos fueron realizados por triplicado.

Resultados / Discusión / Conclusión

Ocho variedades de maíces nativos fueron evaluadas en su actividad antioxidante respecto al maíz híbrido blanco, de estas, tres demostraron mayor contenido de fenoles respecto al maíz híbrido local destacando el maíz tabloncillo harinoso rojo, reventador güino rojo y tabloncillo harinoso negro por 17, 37 y 40 mg/g equivalentes de ácido gálico; a su vez, la actividad antioxidante fue superior en un 10, 39 y 72% en el mismo orden. Se realizaron cultivos de *Lactobacillus acidophilus* en presencia y ausencia de harina de maíz rojo güino, para establecer la adaptabilidad y digestibilidad del microorganismo a la misma. Se encontró que el consumo de azúcares fue similar en el medio MRS, mientras que en el caso de leche entera el consumo fue 1.53 veces superior con harina observándose incluso, el cuajamiento de la misma. En el buffer acetatos se logró un crecimiento pobre en ausencia y presencia de harina. En correspondencia, la producción de ácido láctico fue de 4.5, 5.8 y 2.5 g/L para MRS, leche entera y buffer acetatos con glucosa (en presencia de harina) respectivamente. Destacablemente, la producción de ácido láctico en leche entera adicionada con maíz fue mayor que la obtenida en MRS+maíz por 1.2 veces y, también fue 1.41 veces superior al obtenido en leche entera sin maíz. Finalmente, la actividad antioxidante al final del cultivo es 10% superior con adición de harina (incluso respecto al control de maíz híbrido) en todos los cultivos. En conclusión, el cultivo de *Lactobacillus acidophilus* en presencia de harina de maíz nativo resulta una alternativa viable para el desarrollo de bebidas probióticas lácteas con poder antioxidante, en el caso de bebidas no lácteas se requiere mejorar la adaptabilidad de este microorganismo, puesto que reporta capacidad de actividad α -amilasa.

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Characterization of the antihypertensive effect of a mixture of citroflavonoids

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Introduction

The *Citrus* genus is characterized as one of the largest accumulators of phenolic compounds such as glycosylated flavanones and polymethoxylated flavones [Khan et al., 2014]. Epidemiological evidence and clinical and pre-clinical studies suggest that flavanones have a positive influence in cardio-metabolic parameters, preventing cardiovascular diseases (CVDs) [Chanet et al., 2012, Cassidy et al., 2012]. Previous studies indicate that the sweet orange's major flavanones are hesperidin (H) and naringenin (N) which are particularly concentrated in the peel (albedo and flavedo) rather than the pulp [Khan et al., 2010; Cabañas, 2013]. González-Rivero 2018 reported synergic vasorelaxant effect of H:N mixture (mix-H:N). This effect was endothelium-dependent through increase of nitric oxide in *ex vivo* assay of vascular smooth muscle [González-Rivero, 2018]. The aim of the present study was to determinate acute and subchronic cardiovascular effect through hypotensive ability in hypertensive rats with the proposal to contribute to the growing knowledge about citroflavonoids for the treatment and prevention of CVDs.

Method

The flavonoids were purchased of Sigma Aldrich Co. Blood pressure changes were evaluated using the noninvasive tail cuff system Panlab Harvard Apparatus, LE 5007. Adults spontaneous hypertensive rats (SHR) and Wistar Kyoto rats (WKY; control group) were randomly divided into four groups of six animals each (n=6). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) values were obtained at the beginning of the experiment (T0). Then, for the acute assay the group I and II were orally administered with 161 mg/Kg of the mix-H:N or distilled water (5 mL/kg) respectively, later the SBP, DBP and HR values were obtained at 1, 3, 5, and 7 hours post-administration. In the subchronic assay the group III and IV were orally administered either distilled water or mix-H:N for four weeks respectively and measurements of SBP, DBP and HR were obtained every week. The results were represented by response-time curves and these were expressed as mean \pm standard error of the mean (SEM) of SBP, DBP or HR values. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values less than 0.05 were considered statistically significant respect to control group.

Results / Discussion / Conclusion

On average, acute orally administration of mix-H:N (161 mg/kg) in SHR caused a significant decreased respect to control (WKY group) in the SBP (8.78% and 7.57%) and DBP (12.3% and 11.35%) at the 5 and 7 hours respectively post-administration. Oral administration of mix-H:N (161 mg/kg per day) continuously decreased the SBP and DBP of SHRs during the experimental period. The mix-H:N caused a decreased in the SBP (15.79%) and DBP (11.26%) in the first week, and these effects were maintained during four weeks (SBP: 23.16% and DBP: 19.59%). The heart rate values not were modified in the acute or subchronic experiments. The mix-H:N significantly decreases the systolic and diastolic blood pressure in hypertensive rodents. These data provide an added value to the pharmacological description of the citroflavonoids mixture.

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***Jungia rugosa* Less: Determinación de la Inhibición de la producción de óxido nítrico en macrófagos y ensayo de viabilidad celular**

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Introducción

Jungia rugosa (Asteraceae) es originaria de Bolivia, Ecuador y Perú (Tropicos®). Sus nombres vulgares “matico de la sierra”, “matico de puna”, “matico blanco”, “karamati”, “guayombo”, “trikachi”, y en Ecuador es conocida como “carne humana” o “fompo” (de la Cruz *et al.*, 2006; Criollo y Molina, 2016). Se trata de un arbusto trepador que crece entre 2900 y 3800 m de altitud en lugares húmedos, junto a los arroyos y riachuelos (Pacheco, 2011; Solís, 2014; Cambizaca y Bermeo, 2015). Los extractos de las hojas de *J. rugosa* han sido tradicionalmente utilizados como antiinflamatorios (de la Cruz *et al.*, 2006).

El óxido nítrico es una importante molécula pleiotrópica que media en una amplia gama de procesos fisiológicos y patofisiológicos. Es ampliamente aceptado el papel fisiológico de bajos niveles de óxido nítrico generado por la enzima óxido nítrico sintasa endotelial en el lecho vascular: modula el flujo sanguíneo, reduce la pérdida de fluido y proteínas en las vénulas y la adhesión de leucocitos. Su participación en las respuestas inflamatorias se ha demostrado en un gran número de modelos experimentales y también en enfermedades inflamatorias humanas. Muchas respuestas agudas y crónicas están asociadas con un exceso de producción de óxido nítrico por la enzima óxido nítrico sintasa inducida en diferentes tipos de células, especialmente en macrófagos (Alcaráz y Guillén, 2002).

Método

Se evaluó la actividad antiinflamatoria de un extracto acuoso liofilizado, obtenido a partir de las hojas de *J. rugosa* por infusión. La actividad antiinflamatoria será estudiada utilizando un modelo *in vitro* (producción de óxido nítrico en macrófagos RAW 264.7 (Bas *et al.*, 2007). El rango de concentraciones a evaluar *in vitro*, se determinará previamente mediante el ensayo de viabilidad celular propuesto por Mossman (1983).

Discusión

Se presentan resultados de la acción inhibidora sobre la producción de óxido nítrico en las células RAW 264,7 para el extracto acuso de *Jungia rugosa*.



Los resultados obtenidos podrían probar y validar el uso que la medicina tradicional atribuye a las hojas de *Jungia rugosa* como antiinflamatorio.

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Cytotoxicity of *Ilex paraguariensis* extract and evaluation of their possible preventive effect against cellular damages alcohol-induced

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Introduction

Chronic or excessive alcohol intake is associated to several toxic effects¹. The treatment of alcoholism involves interventions at many levels, and preventive actions are pivotal to minimize damages. Plants have been used as therapeutic alternatives for a long time and *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae) may be a promising alternative²⁻³. Recently TAMURA et al.² demonstrated a protective effect on liver damage caused by ethanol in rats using an extract from toasted herb incorporated into feed. The present work proposes an investigation of the cytotoxicity of *I. paraguariensis* extract, as well its possible preventive effect on alcohol-induced cellular damage.

Method

The extraction procedure was performed in triplicate. For assays, NIH-3T3, AML-12 and PC12 cell lines were used. Cells were maintained in 25cm³ Nunc flasks with DMEM medium supplemented with 10% (v/v) fetal bovine serum and 100 IU/ml penicillin at 37 °C in a humidified atmosphere containing 5% CO₂. When the confluence reached about 50-60%, the cells were seeded into 96-well plates. All incubations with the different concentrations of the extracts and ethanol occurred over a 24-hour. The extract cytotoxicity was measured by assay with tetrazolium salt (MTT). This test quantifies viable cells capable of metabolizing MTT to formazan. The absorbance reading of formazan at 490nm is directly proportional to the number of viable cells. In this way, the IC₅₀ was established. Concentrations in the range of 0.1 to 10mg of extract/mL of cell medium were tested. To verify the possible preventive effect of the extract against the alcohol-induced damage, the extract concentrations were chosen based on the IC₅₀ measured and used different ethanol concentrations (0-10M). In addition to the MTT test, the live/dead cell viability assay was performed. In this test, the calcein-green polyanionic dye is retained within living cells, producing an intense green fluorescence. On the other hand, the ethidium-1 homodimer enters the cells with damaged membranes producing red fluorescence in dead cells. For analysis the samples were photographed using a fluorescence microscope at 100 × magnification. The results were analyzed by one way ANOVA. Differences were considered significant when p<0.05.

Results / Discussion / Conclusion

In order to evaluate the cytotoxicity of the extract, a curve with concentrations of 100, 250, 500, 750 and 1000 μg extract/mL medium was made in the 3 cell lines. However, no significant difference in cell viability was verified in relation to the control. A new assay was carried out, raising the range of concentrations used: 1, 1,5, 2,5, 5 and 10 mg. Considering the values of the control group as a 100% viability, the IC_{50} values of the extract were calculated for all studied cell lines, and good therapeutical safety was verified, aiming to in vitro tests. Using MTT assay, assays for the evaluation of preventive effect of the extract investigation were carried out. First, the ethanol toxicity was evaluated, using the concentrations between of 0 and 10M. The extract was tested in different concentrations against alcohol-induced cytotoxicity for the comparison of the different treatments on cell viability. A positive effect of extract was observed, in which all cell lines completely prevented damage caused by 1M ethanol administration and partially to 2,5M. In the live/dead test, the extract were also tested against alcohol citotoxicity. The preventive effects were in agreement with MTT results, showing a considerable positive effect of the treatment with extracts. In this context, our results indicate that *I. paraguariensis* extract presents a low cytotoxicity by itself, and shows a promising preventive effect against alcohol-induced cellular damages in the experimental model used. (Financial support: Capes, CNPq, FAPESP and FAEPEX-UNICAMP)

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Mallow mucilage (*Malva sylvestris*) in the clarification of agave mead (*Agave americana*)

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Introduction

Agave honey is a product obtained by the concentration of sugars present in the exudate that is produced by scraping the meristem from agave plants, this exudate is also known as mead. The clarification of the mead consists in eliminating the suspended solids that don't constitute sugars and that diminish the quality when generating dark colors in the honey. The aim of this research was to evaluate the clarifying effect of the mallow mucilage in the agave mead in the process of making honey to improve the appearance in terms of color of the final product.

Method

For the clarification of the mead, the mucilage was extracted from thick and tender stems of the mallow in a state of flowering, the bark was removed and it was crushed in order to facilitate the obtaining of the mucilage. Then the plant material was wetted with water, in a 1:10 ratio with constant agitation for five minutes, then left to rest for thirty minutes. Finally it was filtered and incorporated into the mead. For the clarification, two incorporation temperatures and two percentages of mucilage to be used were evaluated, then the mead was allowed to concentrate until reaching approximately 65° Brix, constantly removing the solids dragged by the mucilage. A completely randomized factorial design A x B with two replications was used and turbidity, brix and pH data were recorded before and after the clarification of each of the treatments. In the final product, a honey color analysis of each of the treatments was carried out.

Results / Discussion / Conclusion

The best results for clarification of agave mead were obtained at a temperature of 90 °C and with a percentage of incorporation of the mallow mucilage of 30%, which reported values of 269.50 NTU for turbidity, 6.4 ° Brix and 5.09 for pH. In the final product the coloring of the honey was evaluated and the best

treatment obtained a value of 222.01 mm Pfund that corresponds to a light amber tone, according to the scale of Panoramic Hill Honey Collective that was taken as reference.

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Fermented beverages of Cassava (*Manihot esculenta*) with kéfir and yeast

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Introduction

“Chicha” is an ancestral fermented drink of importance to the aboriginal peoples of the Amazon region of the Ecuador’s communities, for that reason it has been considered the implementation of technologies to obtain fermented beverages from cassava (*Manihot esculenta*) with two kefir fermenting agents and yeast in different concentrations to be able to perform an evaluation of the intrinsic characteristics of the products elaborated in comparison to the white “chicha”, handcrafted artisanally the inhabitants of the Amazon people make through a process of mastication of the cassava pulp where the amylase enzyme acts as a means of degradation by transforming the starch into sugars, meeting the needs of the consumer and with the sensory characteristics of the artisanal “masato” made in the “Madre Tierra” sector, “Puyo” city. The objective of the present investigation was to evaluate the fermentation of cassava (*Manihot esculenta*) subjected to three treatments with kefir and yeast for obtaining fermented beverages.

Method

To make cooked cassava “chicha”, peel and cook in unsalted water until softened. Then the cassava is passed to a wooden pan and crushed with a mallet, this crushed yucca is called “masato”. The next step is to replace the chewing or salivate the pieces of “masato” that have been hard or fibrous. Then the striped sweet potato (*Ipomoea batatas*) was added. For the fermentation of the “masato” it is placed in a clay pot with cane base and lined with “bijao” leaves so that it starts its fermentation process that can last from 3 to 4 days for its consumption, and the maceration with kefir or yeast at different concentrations and then a sieving is done to separate the solid part of the liquid after a subsequent mixing and the drink can be consumed directly by people who are going to perform physical activity. For the fermentation, three “chicha” elaboration processes, two fermenting agents (kefir and yeast) and two concentrations of fermenting agents were evaluated, then the drinks were left to ferment. A factorial design of A * B * C with two repetitions was used and data of ° Brix, turbidity, pH and alcoholic degrees were recorded before and after the fermentation of

each of the treatments. In the final product an analysis of reducing sugars, alcohol content (ethanol and glycerol), viscosity, turbidity and sensory analysis of the best treatment was carried out.

Results / Discussion / Conclusion

The best results for the fermentation of the cassava “chicha” were obtained the white cassava “chicha” with the yeast fermenting agent, which reported pH values, turbidity, °Brix and alcoholic degrees similar to those obtained in the aboriginal peoples of the Amazon region of Ecuador’s communities.

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Síntesis de 2-alcoxiselenazolininas derivados de carbohidratos

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Introducción

La d-glucosamina es un producto natural que se produce en el cuerpo humano, se encuentra como componente celular de numerosas glicoproteínas, glicolípidos, y glicoaminoglicanos. Muchos estudios han demostrado que la d-glucosamina tiene actividad anticancerígena.¹ Por otro lado el funcionamiento anómalo de las hexosaminidasas conduce a la acumulación de glicoconjugados parcialmente degradados,² lo cual origina algunas enfermedades de almacenamiento lisosomal, como el Tay-Sachs, o Sandhoff. Los inhibidores de esta familia de enzimas pueden usarse en el tratamiento de estas enfermedades; entre ellos destacan las tiazolininas bicíclicas derivadas de carbohidratos. La sustitución isostérica del átomo de azufre por selenio podría, por un lado, mantener la actividad inhibitoria, y además conferirle actividad anticancerígena. El objetivo de este trabajo es la preparación de 2-alcoxiselenazolininas bicíclicas derivadas de d-glucosamina, mediante la funcionalización adecuada de ésta.

Método

Se propuso la síntesis de 2-alcoxiselenazolininas utilizando como materia prima asequible el hidrocloreto de la d-glucosamina mediante una protección quimioselectiva del grupo amino en forma de imina, seguido de acetilación convencional de los hidroxilos, y su N-desprotección final en medio ácido. Posteriormente el compuesto se hizo reaccionar con anhídrido acético-fórmico (AFA) para dar la formamida correspondiente, que posteriormente se utilizó para la formación del isoselenocianato utilizando trifosgeno como agente deshidratante seguido de adición *in situ* de Se negro. Subsecuentemente se llevó a cabo el acoplamiento del isoselenocianato con diferentes alcoholes (metanol, etanol, propanol, pentan-1-ol, dodecan-1-ol y octadecan-1-ol), proporcionando los selenocarbamatos correspondientes. A continuación se llevó a cabo la ciclación intramolecular de los selenocarbamatos de metilo, etilo y propilo mediante catálisis ácida (AcOH), dando lugar a la formación de las 2-alcoxiselenazolininas. Finalmente, las 2-alcoxiselenazolininas se hicieron reaccionar en amoníaco metanólico, para la eliminación de los grupos acetilo, conduciendo de esta forma a las 2-alcoxiselenazolininas bicíclicas desprotegidas.

Resultados / Discusión / Conclusión

Se ha accedido de forma sencilla a una serie de selenocarbamatos derivados de d-glucosamina como materias primas clave. Se hizo necesario el uso de 10 equivalentes de AcOH como catalizador para promover la sustitución nucleófila del átomo de Se sobre el carbono anomérico del azúcar, mediante protonación del grupo acetoxi saliente, originando el correspondiente sistema bicíclico. En cuanto a la desprotección, las condiciones óptimas implican el tratamiento con NH₃ metanólico.

Por otra parte, los resultados de actividad antiproliferativa preliminares sugieren una actividad relevante de los selenocarbamatos *O*-protegidos.

Agradecimientos

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Caracterización del aceite obtenido de semillas de *Tectona grandis* de origen Ecuatoriano

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Resumen

Teca (*Tectona grandis*) es una de las diversas especies que se encuentran en Ecuador (Bonilla, 2017), perteneciente a la familia *Verbaseae*, y originaria de los países asiáticos (India, Myanmar, Laos y Tailandia). La especie presenta gran importancia económica siendo su madera utilizada en construcciones estructurales, navales y fabricación de muebles entre otros usos, debido a su resistencia y durabilidad, haciéndola exótica tanto a nivel nacional como internacional.

Este estudio presupone la posibilidad de aprovechar un desecho industrial como las semillas, para obtención de aceites cuyo potencial se desconoce. Es por esto que se ha determinado la composición química y las propiedades fisicoquímicas que posee el aceite obtenido de semillas de la *Tectona grandis*; considerando que de la especie ecuatoriana no se evidencian reportes científicos hasta la fecha.

Por otro lado, las investigaciones sobre aceites de origen vegetal se han incrementado, no solo por la búsqueda de nuevas fuentes de este importante recurso, sino por la demanda de industrias relacionadas con el campo farmacéutico y cosmético.

En este trabajo se evaluó el proceso extracción de aceite de semillas de *T. grandis*, de origen ecuatoriano, para el aprovechamiento de este recurso como residuo agroindustrial. El aceite se obtuvo mediante extracción clásica por soxhlet, comparando el rendimiento del producto en base a diferentes disolventes y condiciones de extracción. Se determinaron las características fisicoquímicas para la evaluación de la calidad del aceite por su densidad, e índices de refracción, saponificación, acidez, yodo y peróxido. El contenido de la fracción saponificable y de la fracción insaponificable fue analizado mediante cromatografía de gases acoplado a espectrometría de masas (GC-MS).

Las condiciones óptimas de extracción evidenciaron un rendimiento del 24% con un CV de 2%, utilizando hexano como extractante a 80-90°C durante 8 horas. La caracterización fisicoquímica demostró que el aceite cumple con los estándares de calidad con potencial interés para la industria, presentando una densidad de



0.92, e índice de refracción de 1.4752. Los resultados cromatográficos indican un importante contenido de ácidos grasos insaturados debido a la presencia de ácido linoleico y ácido oleico; así como también fueron identificados esteroides característicos de la *Tectona* de otros orígenes, como es el caso de sitosterol y stigmasterol.

Palabras clave: *Tectona*, Teca, semilla, ácidos grasos, esteroides

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Bioactivity and profile of phenolic compounds in flowers growing in Brazilian cerrado

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Introduction

Flowers from ancient times have been used as edibles and for medicinal purposes in a very specific way. However, when related to secondary metabolites and bioactivities, there is still little research on phytochemical characterization of this plant structure.¹ Therefore, this work describes studies of chemical research by HPLC-DAD, CE-UV and biological activity of four flowers collected in the Cerrado in northeastern Brazil in order to obtain a metabolic profile that guides the discovery of new metabolites with chemical-pharmacological potential.

Method

The native flower species Cerrado biome *Fridericia platyphylla*, *Turnera ulmifolia* L., *Momordica charantia* L., *Handroanthus albus* (coded S1-S4 respectively) were collected in the city of São Raimundo das Mangabeiras-South of the State of Maranhão. Voucher specimen are preserved in the Graziela Barroso herbarium of the UFPI. Fresh flower samples (0.5 g) were extracted with 10 mL of methanol solvent, filtered, and then concentrated in a rotary evaporator. The obtained extracts were cleaned-up using reverse-phase Sep-Pak cartridges and subsequently subjected to HPLC-DAD and CE-UV analysis. The extracts were again evaluated for their antioxidant (DPPH) and antifungal activity against *C. albicans*, *C. tropicalis* and *C. parapsilosis*.²

Results and Discussion / Conclusion

The chromatographic profiles of flower extracts showed several peaks, including derivatives of phenolic compounds, especially flavonoids with two characteristic absorption bands (240-285 nm band I for ring A and band II 300-550 nm for ring B).³ The methanol extract of species 1 to 4 showed good antioxidant activity for DPPH radical when compared to positive and negative controls. Species S1 and S2 in 0.5 mg/mL reduced the growth of *C. tropicalis* by 47 and to 69%, and of 29 and to 71%, of *C. parapsilosis* respectively. This study contributes to the knowledge of the chemical and biological diversity of the medicinal resources available in the regional flora as well as the biological and pharmacological applications.

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Effectivity of different methods for the extraction of principle actives and phytochemicals content in medicinal herbals

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Introduction

Ageratum conyzoides (AC), *Plantago major* (PM) and *Arctium lappa* L. (AL) are three medicinal plants of enormous phytotherapeutic importance. AC, for instance, is widely used in several cultures as a remedy for a wide range of diseases, possibly due to its excellent anti-inflammatory and antinociceptive properties¹. AL, is a vegetable that has been widely used as a diuretic and for the treatment of hypertension, gout, arteriosclerosis, hepatitis, and others inflammatory disorders². Likewise, several species belonging to the genus *Plantago* (especially PM) has been widely used for decades in the treatment of various ailments, including infections, inflammation, and cancer³. In this present study, we investigated the influence of various extraction methods on the chemical and biological potential of phytochemicals extracted from three medicinal plants (*Ageratum conyzoides*, *Plantago major* and *Arctium lappa* L.).

Method

Several extraction methods were used to obtain the phytochemicals from the three species, including: maceration, sonication, infusion, decoction, and microwave. In order to compare various methods, the results were analyzed by variance analysis.

Results and Discussion / Conclusion

Our results suggested that sonication is the most effective extraction method among the five methods tested herein, for the extraction of phytochemicals that have a high antioxidant potential and high phenolic content. The three plants employed for this study had a high concentration of flavonoids and phenolics, which were compatible with the chemosystematics data. All the samples possessed a sun protection factor (SPF) of less than six. Interestingly, a maximum reaction time of approximately 20 min was noted for the complexation of $AlCl_3$ with the flavonoids present in the phytochemical extract during analyses of the kinetic parameters. *Ageratum conyzoides* extract, prepared by sonication, possessed a significant pharmacological potential against hepatocarcinoma tumour cells, and therefore provides a biological basis for the therapeutic use of *Ageratum conyzoides*.

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***Salvia semiatrata*: medicinal plant used as anxiolytic and analgesic**

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Introduction

Salvia semiatrata Zucc. (Lamiaceae) is distributed in the state of Oaxaca, Mexico, it's a shrub 1 to 2 m high, with purple flowers and flowering period between June-October¹. *S. semiatrata* is used in the community of Santiago Huaucilla, Oaxaca, as a healing, anti-inflammatory, for ear pain, stomach pain and for nervous disorders². Diterpene metabolites such as semiatrin, tilifolidione and horminone³ have been isolated and characterized from this species, which have antibacterial, cytotoxic and antifeedant properties⁴. A previous study showed that the low polarity extracts of *S. semiatrata* have anxiolytic and analgesic properties, therefore, in this work, dose-response curves were made of the hexane and ethyl acetate extracts and the metabolites present in said extracts were identified.

Method

The aerial part of *S. semiatrata* was dried and ground finely (1466.3 g), extracts were obtained with hexane and ethyl acetate, by maceration. For the pharmacological evaluation, male CD-1 mice were used. The evaluation of the anxiolytic effect was carried out using the open field and pluz-maze models at doses of 3, 30 and 300 mg / kg. On the other hand, the estimate of the analgesic effect was evaluated by the abdominal stretching test at doses of 10, 100 and 300 mg / kg. Fractionation of the extracts was carried out by conventional column chromatography on silica gel, with an elution system of increasing polarity (hexane, hexane-ethyl acetate, ethyl acetate-methanol). Fractions of 100 mL were collected, concentrated in a rotoevaporator and analyzed by thin layer chromatography, to collect them by their similarity. The fractions with less complex profiles were purified by crystallization and analyzed by gas chromatography coupled to mass spectrometry (GC-MS), under the methodology of the chemistry research center of the Autonomous University of the State of Morelos.



Results / Discussion / Conclusion

The hexane extract (3, 30 and 300 mg / kg), as well as the ethyl acetate extract at dose of 300 mg / kg showed a significant anxiolytic effect. With respect to the analgesic activity, both extracts reduced the nociceptive behavior in the doses evaluated. The GC-MS analysis of a solid obtained from the hexane extract revealed the presence of 8 compounds, the most abundant being β -sitosterol (72.78%). On the other hand, in a precipitate of the ethyl acetate extract, 12 metabolites (hydrocarbons, fatty acids and triterpenoids) were determined. The fractionation of this extract, also allowed to obtain an oil whose analysis showed the presence of mono-, di-, tri- and sesquiterpenoids, of which, the majority were spatulenol (14.63%), phytol (12.41%) and caryophyllene oxide (11.39%). The study supports the use in traditional medicine of *S. semiatrata*, where metabolites with recognized anxiolytic and analgesic activity were identified: ursolic acid, oleanolic acid, β -amyrin, β -sitosterol, phytol and caryophyllene oxide.

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Preliminary monitoring of heavy metals in *Sparus aurata* L. and *Sarpa salpa* L. from Tunisia

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Introduction

The presence of heavy metals in human tissues is not surprising since the food may be contaminated by As, Cd, Cr, Hg and Pb. These elements can accumulate in the food chain and may reach dangerous levels for human health. Due to high concentrations of these five elements in marine fish and their potential harmful effects to consumers, the concentration of heavy metals in two fish species: *Sparus aurata* L. and *Sarpa salpa* L. collected from the coast of Mahdia (Mediterranean Sea, Northern Tunisia) was evaluated. The studied species in this research are common in the five Tunisian sites considered and are usually favoured by local consumers.

Method

A total of 80 fish samples were collected in the year 2019. Approximately 10 g of fish muscles were ground with a teflon mortar to avoid metal contamination. About 0.5 g of each sample were accurately weighed into acid-pretreated PTFE vessels, add with 1ml of Re internal standard, and then were digested with 10 ml HNO₃ using a closed-vessel microwave digestion system.

After microwave digestion, metal concentrations were determined by ICP-MS. Method validation was carried out according to EURACHEM criteria. All samples were analysed in batches, with blank samples and known standards. Each analysis was carried out in triplicate.

Results / Discussion / Conclusion

In *Sparus aurata* the concentrations ranged from 13.99 to 1367.85 µg/kg for Cr, 66.84-11.75 µg/kg for As, 0.16- 3.87 µg/kg for Cd, < LOQ -56.56 µg/kg for Pb, and 74.73- 675.41 µg/kg for Hg; in *Sarpasalpa* the concentrations ranged from 32.60 to 193.89 µg/kg for Cr, 127.13-4847.51 µg/kg for As, < LOQ - 2.14 µg/kg



for Cd, < LOQ to 0.04 µg/kg for Pb, and 45.34 to 684.58 µg/kg for Hg. Arsenic, mercury and chromium content detected in *Sparus aurata* is higher than the limit fixed by the European legislation (360 µg/kg; 500 µg/kg; 200 µg/kg respectively), while they are less in *Sarpa salpa*. Cd and Pb were always below the limits (50 µg/kg for cadmium and 200 µg/kg for lead respectively) (EC, 2006, 2008). To our knowledge, this is the first study entirely focused on heavy metals concentrations in *Sparus aurata* L. and *Sarpa salpa* L. from Mahdia coast (Tunisia).

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Preliminary study on plasticizers and BPA content in honey samples from Algeria

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Introduction

Honey has always been considered a natural and healthy food; for this reason, it requires standards that guarantee its quality so that consumers may safely eat it. Many studies on honey contaminants, such as metals, pesticides, PAHs and antibiotics have been carried out, but few studies have been conducted on the presence of plasticizers and bisphenol A. Today, the migration from plastic packaging is considered as among the major source of human exposure to these compounds, which however could also result from pollutants of the marine and terrestrial environment and/or agriculture practice. The aim of this preliminary study was to investigate the content of plasticizers and BPA in honey samples produced in Algeria, in order to characterize their actual contamination status.

Method

Twenty-nine multifloral honey samples produced in different regions of Algeria (Algeri, Laghouat, Ghardaia, El-Bayadh, M'sila, Bouira, Tlemcen, Naama) in 2018 were analyzed. All the samples were provided directly by local beekeepers and packaged in glass jars. The extraction from honeys was conducted by Micro-Matrix Solid Phase Dispersion, using Florisil and ethyl acetate for elution. The residues of 20 plasticizers (phthalates, adipates, sebacates, and others) and of BPA were investigated by gas chromatograph equipped with quadrupole mass spectrometer. This analysis was performed in Single Ion Monitoring (SIM), using three characteristic mass fragments for each analyte (the first one as the target ion and the rest two as the qualifying ions). In order to evaluate the procedure for the analytical protocol, this method was validated according to the European Union guidelines.

Results / Discussion / Conclusion

DMA, DEA, DiBA, BB, BPA, BBP, DiHpP, DcHeP, DPhP, DEHS, DiNP and DiDP were always lower than their analytical LOQ in all samples; DBA, DBP and DEHA residues were found in some ones. Conversely, all honeys contained DMP, DEP, DPrP, DiBP, DEHP and DEHT. Generally, DEHT was the most abundant

plasticiser from 1.13 to 81.14 mg/kg, followed by DEHP with a mean concentration of 0.74 mg/kg and DiBP (0.28 mg/kg). Since these plasticizers can be detected in the plastic components of the equipment for the honey production, such as honey extractor or uncappers, it is possible assume that the product can be contaminated by these compounds during the beekeeping steps. Moreover the contaminated nectar can be also a source of these plasticizers, as a result from environmental pollution (air, water and/or soil) and/or agricultural practices.

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Silkworm larvae (*Bombyx mori*) proteins in health

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Introduction

Protein malnutrition in Mexico is a problem that should be examined within the whole context of the national socio-economic milieu. Proteins are an essential macronutrient humans require to have adequate cellular and organ functions. The diet must contain not only enough protein and amino acids, but also sufficient not protein energy to permit optimal utilization of dietary proteins. Insects consumption is a cultural tradition for Mexican population, this small animals provide macronutrients, including proteins with all essential amino acids. This study was conducted to assess macronutrient composition of the larvae of *Bombyx mori* silkworm edible insect that can help to improve health.

Method

Silkworms were obtained at Hidalgo State in Mexico, late spring 2018 and *Bombyx mori* larvae, analyzed in dry basis according AOAC (1995) methods to determine, proteins, lipids, total minerals, fiber and soluble carbohydrates.

Results / Discussion / Conclusion

Data obtain was: proteins 52.07%; lipids 29.82%; minerals 5.24%; fiber 0.86%; soluble carbohydrates 12.01%. Protein deficiency has adverse effects in all organs and on particular concern may have long term consequences on brain functions. Silkworm edible insect will represent a valuable source of proteins in a diet, and plays a crucial role for the support of the body physiological and organs systems for a good nutrition and health.

Proteínas de chía (*Salvia hispanica*): nuevas moléculas para la estabilización de emulsiones aceite en agua (O/W)

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Introducción

Las semillas de chía han cobrado gran relevancia en los últimos años debido a su alto valor nutricional. Particularmente, el contenido proteico es de resaltar, siendo superior al de la mayoría de los cereales de mayor consumo (19-23% p/p)¹. Por otro lado, las emulsiones aceite en agua (O/W) son parte integral de muchos productos alimenticios, estos son sistemas termodinámicamente inestables y requieren la adición de emulsificantes para extender su vida útil. Gran parte de los emulsificantes utilizados en la industria son de origen sintético o animal, generando controversia entre los consumidores². Las proteínas de chía pueden ser excelentes sustitutos de estos materiales debido a su naturaleza anfifílica, lo cual permitiría generar productos de alto valor agregado al fusionar las cualidades nutricionales y tecnológicas de dichas moléculas³. Por lo tanto, el objetivo de este trabajo fue evaluar la capacidad de estas proteínas para formar emulsiones O/W y analizar los mecanismos que causan inestabilidad física en estos sistemas.

Método

a) Cuatro fracciones proteicas con distinto grado de hidrofobicidad (HO) fueron solubilizadas en buffer de fosfatos (50 mM, pH 7) hasta una concentración de 1% (p/v). Se usó un medidor de ángulo de contacto óptico (OCA-20) con accesorio de caída oscilante ODG-20 (Dataphysics Instruments GmbH, Alemania) para analizar la dinámica de adsorción de cada una de las fracciones, monitoreando la evolución de la tensión interfacial a través del tiempo en una interfaz agua-aceite durante 90 minutos. El aceite utilizado en este análisis fue aceite de canola filtrado (0.2 μ m).

b) Se prepararon emulsiones O/W con cada fracción proteica usando dos métodos de producción: rotor-estator (R-E) y ultrasonido (US); además, se analizó la influencia de la concentración de la fase dispersa (ϕ) (3% p/v y 5% p/v). La estabilidad física se evaluó mediante una centrifuga analítica LUMiSizer, L.U.M. GmbH, Berlín, Alemania).

Resultados / Discusión / Conclusión

La fracción con el mayor grado de HO fue la más eficiente para disminuir la tensión interfacial (TI), una clara correlación entre el nivel de HO y la capacidad para disminuir TI fue observada. Al elaborar las emulsiones, se demostró que este hecho favorece la generación de tamaños de gota inferiores a 1 μm al utilizar US y de 1.4 μm con R-E como método de fabricación, independientemente de la ϕ utilizada, creando así sistemas más estables. El análisis de los perfiles de transmisión obtenidos con el LUMiSizer reveló que el cremado y la sedimentación fueron los principales fenómenos asociados a la inestabilidad de las emulsiones. De esta manera, se verificó que altos valores de HO favorecen la estabilidad de emulsiones O/W, no obstante, niveles muy elevados pueden conducir a fenómenos de agregación e inestabilidad². Así mismo, los resultados obtenidos sugieren que las proteínas de chíá pueden ser sustitutos adecuados de emulsificantes utilizados ampliamente en la industria, como las proteínas de soya, ofreciendo resultados incluso superiores a estas². Por lo tanto, dichas moléculas representan una opción altamente viable en la búsqueda continua dentro del sector alimentario por encontrar ingredientes altamente versátiles y funcionales, en este caso, combinando las propiedades nutricionales y tensoactivas de las proteínas de chíá.

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Multi heart-cutting two-dimensional liquid chromatography coupled with tandem mass spectrometry detection for determination of mycotoxins in beer

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Introduction

Beer is one the most consumed alcoholic beverage in the world and its contamination with mycotoxins is of public health concern. This study report the development and validation of a fast and automated analytical procedure based on a multi-heart-cutting two-dimensional liquid chromatography tandem mass spectrometry method using electrospray ionization for the determination of seven mycotoxins (aflatoxins, ochratoxin A, fumonisins) in beers.

Method

The developed method was based on the heart-cutting 2D- HPLC technique in which only the specific portions of the first dimension, in the retention time of analytes, were transferred into the second dimension for the further separation and successive determination. The method uses two different chromatographic columns; in the first dimension, 50 μ L of sample was injected on first column, and mycotoxins elution regions were collected in a loop and transferred into the second column for the separation of analytes. Each column operated in gradient elution mode in order to eliminate interfering compounds and improve separation and peak shape. After the optimization, the method has been validated according to EU regulation and finally applied for the analysis of forty beer samples collected from Italian supermarkets.

Conclusion

Among all analysed samples, fumonisins B1 was widely distributed in beers (>25%) in the range from 0.6–to 12.3 ng mL⁻¹. The automated methodology developed was able to determine accurately and simultaneously seven mycotoxins in beer. This provided a significant reduction of sample handle and, consequently of analysis time.

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Comparación fitoquímica de plantas hipoglucemiante usadas en la medicina tradicional veracruzana, México

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Introducción

Diversos estudios etnobotánicos han demostrado que la población humana continúa usando las plantas medicinales para tratar algunas enfermedades afecciones, entre los que se destacan especies de plantas para controlar los niveles de glucosa en sangre. En Veracruz, México esta práctica es considerada como unos de los remedios caseros importantes en el cuidado de la salud, por su bajo costo, disponibilidad, su gran valor en el conocimiento tradicional y cultural. Desde punto de vista químico, se ha dejado aún lado la investigación química del uso y forma de preparación tradicional. Generalmente, las poblaciones aledañas a las ciudades, colocan la (s) parte (s) de la planta en cocción con agua, y se toman el agua con las hierbas, o bien se hacen lavados con ella, y dependiendo del uso terapéutico es el tiempo de consumo o lavado. Considerando y rescatando el uso tradicional, el principal objetivo de este estudio fue comparar el tipo de extracto acuoso y etanólico de dos especies comunes *Justicia pectoralis* Jacq. y *Salvia úrica* Eplig, con actividad hipoglucemiante usadas en Veracruz, Mexico.

Método

Se colectaron hojas frescas de la especie *Justicia pectoralis* Jacq. y *Salvia úrica* Eplig, en la localidad de Juchique de Ferrer y Xico, Ver, Méx. Posteriormente las hojas se pesaron previamente y se colocaron en un contenedor con agua para obtener el extracto acuoso. Para ello se coloraron en una estufa y se deje hervir durante 5 minutos. Para la obtención del extracto etanólico, se obtuvo mediante maceración de 48 horas; ambos extractos se llevaron hasta sequedad. Se utilizo una prueba colorimetría, cromatografía de gases y

cromatografía de líquidos acoplados a detectores de espectrometría masas para determinar el perfil fitoquímico de ambos extractos.

Resultados / Discusión / Conclusión

Mediante la prueba fitoquímica colorimétrica de ambas especies se encontraron metabolitos secundarios una mayor presencia de flavonoides, alcaloides y terpenos o esteroides; y menor presencia de cumarinas. Asimismo, se encontró ácidos fenólicos, tales como ácido gálico y cafeico. A través de las cromatografías de gases se detectó compuestos mayoritarios como el (Z,E)-farnesol, el Cycloisolongifolene, 7-bromo- y el 1,3-dioxolan-2-one, 3-methyl-3-(4,8-dimethylnona-3,7-dienyl)-4-methylene, estos compuestos han sido encontrados en aceites esenciales y extractos de plantas de la familia Lamiaceae y plantas descritas con propiedades antidiabéticas (Venkataramani y Chinnagounder, 2012). Ambas especies, *Justicia pectoralis* Jacq. y *Salvia úrica* Eplig, presentan compuestos que podrían jugar un papel importante para controlar los altos niveles de glucosa sanguínea, considerado una enfermedad conocida como la diabetes que ha causado muchos problemas a nivel mundial (Correa y Alcantara, 2012).

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Rapid determination of chloramphenicol in honey using salt-induced liquid–liquid extraction coupled with ultrahigh performance liquid chromatography tandem mass spectrometry detection

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Introduction

Honey is a food produced from honey bee, widely used for its sweetening power and nutritional value. In addition to containing nutrients, honey may carry toxic chemical contaminants, such as pesticides and antibiotics. Antibiotics, in particular, are used by beekeepers to prevent or treat hive infections from various pathogens. One of these substances is chloramphenicol, a bacteriostatic antibiotic with a broad spectrum of antibacterial activity. The chloramphenicol's toxic effects are well known and consist of severe forms of agranulocytosis and aplastic anaemia. Because of its toxicity, the use of chloramphenicol was banned in products of animal origin both in Europe and in other countries of the world. Although, it is still illegally used. For this reason, there is a growing need for rapid, simple and accurate analytical methods that allow to quantify the veterinary drugs present in foods, reduce costs and, at the same time, increase the number of samples analysed.

Method

The aim of this study was to develop and validate a salt induced liquid-liquid extraction (SILLE) method coupled with UHPLC MS/MS determination, for fast and accurate analysis of chloramphenicol in honey. Salt induced liquid–liquid extraction is a variant of the classic liquid–liquid extraction, that uses water-miscible extracting solvent and is based on the separation of this solvent from aqueous solutions in the presence of high concentrations of an appropriate salt. The influence of several parameters on the extraction efficiency, such as nature of the extraction solvent, pH of honey sample solution and effect of salting-out were preliminary investigated before performing their optimization by the experiment design. Afterwards, a Box-Behnken design was used to evaluate the influence of four independent variables on the extraction efficiency. Once extracted, chloramphenicol was determined by Ultra High Performance Liquid Chromatography tandem Mass Spectrometry.

Conclusion



Under optimal conditions, detection was linear in the concentration range of 0.4–100 ng mL⁻¹ with a correlation coefficient of 0.9968. The detection limit was 0.09 ng mL⁻¹ and the relative standard deviations ranged between 0.8 and 5.3% (n = 5). The applicability of the developed method was demonstrated for the analysis of chloramphenicol in different honey samples and the relative recoveries ranged from 90.4 to 98.5%. The present method has proven to be simple, sensitive, less organic solvent consuming, inexpensive and rapid for the analysis of chloramphenicol. The speed and easiness of the extraction technique combined with the high sensitivity and accuracy of the detection method are the main advantages of the proposed procedure.

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Identification of antioxidant genes from fungal transcriptomes by using bioinformatics strategies on High Performance Computing (HPC) environment

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Introduction

Fungi produce a great variety of secondary metabolites in the life cycle, which usually occurs in the relations with the hosts. It has been shown that the ability of fungi to overcome the defense mechanisms of the host, in general, is related in large part to the production of toxins, peptides, proteins and others molecules that offer important advantages. In addition, these metabolites also have antimicrobial, anticancer, and antioxidant properties with high bioaccumulation potential (Thakur and Smith, 1997, Nilanonta et al, 2000 Koul and Dev, 1997). The use of genomic and bioinformatics tools allow improving the strategies of bioprospecting In Silico for the identification of genes, proteins and metabolites as a contribution to the generation of alternatives for the treatment of different diseases that affect the population. In order to identify antioxidant potential genes several basidiomycetes were studied by using molecular, biotechnological and bioinformatics strategies.

Methods

Specimens were collected and preserved for identification through taxonomic keys; then, after DNA extraction, it were corroborated by PCR using the primers ITS5 and ITS 4 described by Toju et al., (2012). For phylogenetic analyzes, the sequences were aligned using the CLUSTAL W program, and the phylogeny was constructed using the MEGA 6.0 program. Total RNA was extracted with the InviTrap® RNA Kit (<http://www.strattec.com/en/molecular>), then, RNA libraries were prepared using the TruSeq RNA preparation kit (Illumina) and the sequencing was performed during 100 cycles (paired-end reads) by using Illumina HiSeq 2000 platform (Zipfel et al., 2004). The transcriptome assembly was performed using Trinity-RNA-Seq version v.2.6.5. Using the TransDecoder version 3.0.0 tool, contigs and aminoacid ORFs were generated. Finally, a purified database of antioxidant profiles in FASTA format and HMMs was built.



Results / Discussion / Conclusion

The present transcriptomic study from basidiomycetes fungi sequencing; initially generated a total of 119.128.602 raw of Illumina reads and after the assembly process using Trinity tool, 127.522 contigs were generated, corresponding to 22.890 proteins that were predicted by the TransDecoder bioinformatic tool. Using UniProt public databases (Protein sequences (The UniProt Consortium, 2015) and Pfam (Domain profiles (Finn, 2014), Blast P tools (alignment and comparison of sequences (Jacob, 2008)) and Hmmer (search of profiles using strategies of hidden Markov models (Söding, 2005), respectively in each database, and after the cross-referencing of each strategy results, were identified genes and proteins with antioxidant potential. It allowed a better approach at the molecular level from fungi under study. Finally, the importance of antioxidant new sources lies mainly in the fact that these sources play a fundamental role in the maintenance of redox homeostasis preventing the development of diseases due to oxidative stress.

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Anti-CHIKV activity of essential oils of Colombian plants belonging to the Verbenaceae, Piperaceae, Poaceae and Labiatae families

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Introduction. The chikungunya virus (CHIKV) is an alphavirus transmitted by arthropods, members of the *Togaviridae* family, are viruses enveloped with RNA genome. CHIKV causes an acute illness in humans, characterized by a triad of fever, arthralgia and maculopapular rash followed by a persistent incapacitating polyarthrititis that can seriously incapacitate the patient for weeks to several months. At present there are no licensed vaccines or effective antivirals approved most of the treatment regimens are symptomatic and based on the clinical manifestations. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by distillation. There is evidence that shows that essential oils from various herb species can directly inactivate the viral particle. The inactivation of the viral envelope by the essential oil and subsequent inhibition of viral replication *in vitro* (herpes simplex virus, hepatitis B virus and human immunodeficiency virus) is observed by incubation of the virus with essential oil before cell adsorption. Only few reports describe the inhibition of viral replication for essential oils and their major compounds. The goal of the present study is the evaluation of the antiviral activity of twenty seven essential oils from plants of the families Verbenaceae, Piperaceae, Lamiaceae (Labiatae), Zingiberaceae, Rutaceae, Poaceae, against an isolated CHIKV for incubation of the virus with essential oil before cell adsorption.

Method. The antiviral activity of essential oils on Vero cells against one TCID₅₀ of CHIKV were carried out using the end-point titration technique (EPTT). Antiviral activity- two-fold dilutions of the essential oils (12.5–100 µg/mL) and viral suspension of one TCID₅₀/ 0.1mL were mixed and incubated for 15 minutes at room temperature before they were added to confluent monolayer Vero E6 cells then it was incubated at 37 ° C in a humidified 5% CO₂ atmosphere for 60 h. Finally, medium was removed and cell monolayers were fixed with a solution of formaldehy (3.5%) and crystal violet (0.4%).

Results / Discussion. In this study we found that the essential oils of *Lippia alba*, *Lepechinia salvifolia*, *Lepechinia vulcanicola*, *Lepechinia bullata*, *Lepechinia betonicifolia*, *Mintostachis mollis*, *Piper bogotensis* and *Thymus vulgaris* with more antiviral activity showed a reduction factor (*Rf*: ratio of the virus titer in the absence over virus titer in the presence of the tested compound) of the viral titer between 1 x 10² and 1 x

10^1 in non-cytotoxic concentrations $\leq 100 \mu\text{g/mL}$. The antiherpetic activity (a DNA virus) and / or antifungal activity of some of these essential oils have been reported. It is possible that the antiviral effect is the result of direct inactivation of the virus (virucidal effect), rather than interference with intracellular stages of the viral cycle (antiviral); due to the alteration of the viral membrane or envelope by interaction with components of lipophilic composition of the oils.

Conclusion. We suggest that these essential oils have potential as broad-spectrum antivirals, against viruses enveloped with DNA and RNA genomes. Future studies will be directed to the evaluation antiviral activity in pre-infective stages of *Lepechinia salvifolia* oil, which showed relevant anti-chikv activity in a concentration of $25 \mu\text{g} / \text{mL}$, for to be determined whether the inhibitory effect of this essential oil is due to binding of the essential oil to the viral particle or viral proteins involved in adsorption and penetration to host cell.

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Trace elements in muscle and digestive gland of red swamp crayfish (*Procambarus clarkii*) in Campania Region (Italy): preliminary observations

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Introduction

The red swamp crayfish (*Procambarus clarkii*) is one of the most diffuse crayfish species in the world also because it is invasive and tolerant to the adverse environmental conditions. *P. clarkii* has been used as indicator species to monitor the environmental quality and the contamination of the biological habitat in previous bioaccumulation studies [1]. Cadmium, lead, mercury and arsenic are non essential elements and can create adverse effects on animal and human health due to their potential toxicity and bioaccumulation in food chain. On the contrary, essential elements, needed for biological activities in trace amounts, but may have toxic effects at higher concentrations. The objectives of the present study were to evaluate the total concentrations of essential (Cu, Se, Cr, Fe, Mn, Ni) and non-essential (Hg, Cd, Pb and As) elements in muscle and digestive gland of *Procambarus clarkii* collected from two different sites of Campania region (Italy).

Method

Samples (n=60) of red swamp crayfish (*P. clarkii*) were caught during summer 2017 in two sites of Campania region (Italy), at Villa Literno, near the Volturno River (n=30) and Sessa Aurunca, near the Garigliano River (n=30). After capture, the specimens were weighed and the total lengths were measured. Then they were frozen at -20°C until the analysis. The abdominal muscle and the digestive gland from each animal was separated and homogenized. Each sample was digested in ultrapure 65% HNO₃ and H₂O₂ in a microwave digestion system. Concentrations of trace elements were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) technique using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT.

Results / Discussion / Conclusion

Mean concentrations of essential and non-essential elements in muscle and digestive gland of *Procambarus clarkii* are summarized in Table 1.

Site	Sample	As	Cr	Pb	Cd	Hg	Co	Cu	Mn	Fe	Ni	Se
Villa Literno	Digestive gland	8.53 4 ±1.5 39	0.03 1 ±0.0 06	0.00 5 ±0.0 11	0.00 5 ±0.0 01	<Lo Q -	0.05 5 ±0.0 10	8.57 7 ±2.1 95	4.79 2 ±2.4 02	8.590 ±8.59 0	0.17 4 ±0.0 46	0.17 4 ±0.0 11
	Muscle	0.62 7 ±0.4 24	1.59 0 ±0.8 94	<Lo Q -	<Lo Q -	<Lo Q -	0.02 1 ±0.0 02	5.17 2 ±1.1 02	0.70 2 ±0.2 55	3.540 ±1.27 7	0.67 0 ±0.2 89	0.10 0 ±0.0 10
SessaAauru nca	Digestive gland	3.24 8 ±1.4 82	0.04 2 ±0.0 39	<Lo Q -	0.00 3 ±0.0 01	<Lo Q -	0.01 4 ±0.0 15	11.5 12 ±3.0 34	13.1 02 ±2.4 85	23.36 3 ±10.4 62	0.06 1 ±0.0 13	0.09 7 ±0.0 14
	Muscle	0.80 8 ±0.5 30	1.54 4 ±0.6 46	<Lo Q -	<Lo Q -	<Lo Q -	0.02 0 ±0.0 04	4.51 8 ±1.1 30	3.80 0 ±2.2 05	6.975 ±2.94 1	0.56 9 ±0.1 45	0.10 0 ±0.0 28

Table 1. Mean concentration (mg/kg w.w.) of trace elements in *Procambarus clarkii* and their standard deviations. LoQ = Limit of quantitation.

The results obtained in the current study show low levels of Cd, Pb and Hg in all samples analyzed and largely below the maximum levels established by the European Commission for muscle meat of crustaceans (Reg CE 1881/2006) and were indicative of low risk for human consumption. The highest concentration of metals was detected in the digestive gland of *P. clarkii* from Villa Literno. Lead, cadmium and mercury showed lower concentration than those reported by other studies [1, 2, 3]. The concentrations of all other elements were also generally lower or similar to those detected by the other authors, except for As and Cr, whose mean concentrations were higher in the present study than in others [2, 3]. Ongoing studies on trace elements in a greater number of *P. clarkii*, in other biological and environmental samples and in other geographical areas, will provide more information on the role of this species as indicator of environmental contamination.

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Cytotoxic Properties and Phytochemical Study of Methanolic Extract from *Baccharis obtusifolia*

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Introduction

Baccharis is the largest genus in the family Asteraceae, with over 500 species distributed throughout North and South America (Abad, 2007). The largest American genus *Baccharis* (Asteraceae) includes about 400 species. Of these species, 20% are locally used for medical purposes or, to a lesser extent, as food or as raw material for different local industries (Abad, et. al, 2006). The most prominent biomedical applications of the *Baccharis obtusifolia* H.B.K., commonly known as “Chilca redonda” include the treatment of rheumatism, liver disease, wounds, and ulcers (Abad, 2007).

Method

The leaves from the *Baccharis obtusifolia* species were collected on Villonaco (mountain of the Loja Province of Ecuador). The collected leaves were subjected to a dehydration, to obtain the extract; we used 145g of dried leaves and cold methanol (4-5°C) by dynamic maceration for 5 hours in a light-free environment. The methanolic extract (8.74g) was filtrated to remove chlorophylls with a reverse phase silica gel RP-18, 6.5 cm. To separate the components from the extract without chlorophylls (fraction 1), a silica gel-60 F254 chromatography column was used. Mixtures of hexane and ethyl acetate solvents were used in polarity starting with hexane (100%) to separate the compounds. NMR and MS were used to characterize and identify the secondary metabolites.

The MTS cell viability assay was used to assess the inhibitory effects of the extracts on the survival of human cancer cell lines: PC-3, RKO, D-384, and MCF-7.

Results / Discussion / Conclusion

From fraction 1, two compounds were identified sakuranetin (5,4'- dihydroxy-7-methoxyflavanone) (**1**), and the 5-hydroxy- 7,4'-dimethoxyflavone (**2**). The effect of the methanolic extract on cell viability was determined via an MTS assay using human cancer cell lines treated for 48 h with the whole extract of *B.*

obtusifolia (50 µg/ml). The extract exhibited a strong inhibitory effect on RKO 89.2%, as in D-384 cells with 60.6%, however, the MCF-7 and PC3 cell lines exhibited moderate inhibitory activity with 20.2 and 18.3%, respectively. Compounds **1** and **2** presented a higher CI_{50} to 100 µM for MCF-7, D-384, PC-3 cell lines; for the RKO cell line we obtained a CI_{50} of 68.11 µM and 34.30 µM for compounds **1** and **2**, respectively. Compounds **1** and **2** were specific for the RKO line, and compound **2** was more effective than compound **1**. Numerous studies have suggested that flavonoids may play a protective role in the prevention of cancer, coronary heart diseases, bone loss, and many other age-related diseases (Cartaya, 2001). The activity found in the methanol extract may be due to the presence of isolated flavonoids. Additional studies of the molecular mechanisms underlying the effect of these secondary metabolites on cancer cell survival are accordingly necessary.

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Molecular networking based-analysis and assessment of total flavonoid content of *Byrsonima intermedia*

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Introduction

The qualitative chemical profile by mass spectrometry may be a more adequate and rapid approach for the establishment of *Byrsonima intermedia* extract fingerprinting. The data analysis by the GNPS platform (Wang et al., 2016) allows a faster identification proposal, due to the similarity grouping of the molecular ions and their fragments.

Method

Leaves of *B. intermedia* were collected at the Municipal Botanical Garden of Bauru - SP (22°20'36''S and 49°01'02''W, altitude of 546 m). The harvesting was performed every two months in one year (2017) to study seasonal variability of secondary metabolites. They were dried at 40°C and pulverized using a grinder. 1 g of powder was extracted with 10 mL of Ethanol 70% (v/v) in an ultrasound bath, three times for 20 min. The concentration of total flavonoids was determined based on the methodology of Sobrinho et al., 2008. The quercetin standard was used in the construction of the analytical curve (250-3.91 µg.mL⁻¹). The extracts of *B. intermedia* were solubilized in ethanol at a concentration of 2 mg.mL⁻¹. The chemical profile of the extracts was obtained by HPLC-ESI-MS/MS analysis and the data were entered in the GNPS platform following the workflow to generate the molecular networking.

Results / Discussion / Conclusion

The data analyzed in the GNPS platform for extracts of *B. intermedia* generated a molecular networking with 932 nodes and 45 clusters. The analysis of the fragmentation pattern of the ions of the protonated molecules according to literature data allowed to record 22 compounds in 70% EtOH extract of *B. intermedia*: phenolic acids (galloylquinic acid, digalloylquinic acid, trigalloylquinic acid, tetragalloylquinic acid [*epi*]catechin, trimer [*epi*]catechin, dimer gallate [*epi*]catechin, dimer digalate [*epi*]catechin) and flavonoids (methoxyquercetin-*O*-pentose, quercetin-*O*-hexose-deoxyhexose, galloyl quercetin-*O*-hexose, quercetin-*O*-deoxyhexose-pentose, quercetin-*O*-pentose, quercetin-*O*-hexose-deoxyhexose, galloyl quercetin-*O*-pentose).

Total flavonoid content ranged from 25.08 to 43.36 mg.g⁻¹ (extract). *B. intermedia* has a very complex composition with phenolic acids, flavonoids and procyanidins and is subject to several environmental factors such as high and low temperature, drought, rainfall and UV stress. The samples harvested in the winter were the ones that presented greater variation in the chemical composition and mainly in the flavonoid content which may be related to abiotic factors that influence the chemical composition in these specie.

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POR

Cacalol acetate inhibit the inflammatory process through changes in the NF- κ B signaling route activated by lps

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Introduction

Many chronic degenerative diseases are characterized by inflammatory processes. The transcription factor NF- κ B (p50 / p65) is one of the main therapeutic targets in these diseases. This factor induces the transcription of important inflammatory mediators (cytokines, adhesion molecules, among others) (Abiodun *et al.*, 2016; Fullerton *et al.*, 2016). In the search for new therapeutic substances, there are several alternatives, such as, medicinal plants. The medicinal plants had been the source of many pharmacological compounds. *Psacalium descompositum* has been considered a vegetal species with high therapeutic potential. From this plant, Cacalol has been isolated and this compound has a good potential in inflammatory diseases treatment. This compound was acetyl to make it Cacalol acetate (a more stable compound) (Jimenez-Estrada *et al.*, 2006). Cacalol is considered responsible for anti-inflammatory effect of this plant. However, its mechanism of action has not been explored. The aim in this research was to establish whether the anti-inflammatory activity of cacalol acetate involves the inhibition of the NF- κ B pathway in vitro.

Method

RAW 264.7 macrophages and HUVEC cells were cultured, pretreated with cacalol acetate and then stimulated with LPS. The proteins of the NF- κ B pathway (p65, I κ B- α , IKK α) and some adhesion molecules (V-CAM and I-CAM) were evaluated by means of Western Blot and the relative expression of cytokines (TNF- α , IL -6 and IL-1 β) were quantified by qRT-PCR.

Results / Discussion / Conclusion

In the Western Blot, a decrease in the levels of I κ B- α phosphorylation and p65 subunit was observed in the groups that had been pretreated with cacalol acetate and then stimulated with LPS, but no phosphorylation

changes were observed at the complex level $IKK\alpha / \beta$. These results were similar in the two cell lines and suggest that the action of cacalol acetate could inhibit the $I\kappa B-\alpha$ phosphorylation rate, which prevents the phosphorylation of p65 and with this the decrease in NF- κB translocation to nucleus. These effects may be associated with the decrease observed in the mRNA expression levels of cytokines; TNF- α , IL-6 and IL-1 β in the RAW264.7 cells, as with the decrease in the adhesion molecules V-CAM and I-CAM production in HUVEC cells. It is important to continue evaluating the factors involved in the inhibit effect of cacalol acetate, as well as other transcription factors involved in the anti-inflammatory action of this compound. Cacalol acetate could be model molecule to the design of new antiinflammatory compounds.

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Chemical, enantioselective and sensory analysis of the volatile fraction of the Ecuadorian species *Myrcianthes myrsinoides* (Kunth) Grifo and *Myrcia mollis* (Kunth) DC.

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Introduction

Many families of the vegetable kingdom among the main Myrtaceae, Asteraceae, Anacardiaceae, Apiaceae, Lauraceae, and Rutaceae are producers of essential oils, whose function is defined in aroma and flavor (Romero, 2004). *Myrcianthes myrsinoides* and *Myrcia mollis* are found in the Myrtaceae family and are found in the province of Loja, south of Ecuador (Aguilar, 2009, Jorgensen & León, 1999).

The chemical analysis of essential oils is carried out by gas chromatography (Ricaldi & Martínez, 2014). The aim of this technique was to characterize qualitatively, quantitatively, enantiomerically and sensorially the essential oil corresponding to the two aforementioned plant species. At the same time that inhibition activity against cholinesterase enzymes was studied.

Method

M. myrsinoides and *M. mollis* were collected in Gonzanamá canton, province of Loja. Their essential oil was obtained by steam distillation for 4 hours, collecting 6 ml, 6 ml, 1.8 ml, and 1.2 ml for *M. myrsinoides* and 4.6 ml for *M. mollis*.

The qualitative analysis was performed comparing lineal retention indices and mass spectra of the constituents of the essential oil with data present in the literature. The quantitative analysis was performed calculating the relative retention factor of each compound according to the combustion enthalpy (Tissot, Rochat, Debonneville, & Chaintreau, 2012).



Furthermore, enantioselective analysis was carried out by means of two enantioselective columns: diethyl tertbutylsilyl-BETA-cyclodextrin and diacetyl tertbutylsilyl-BETA-cyclodextrin. The enantiomeric elution order was determined by previous injection enantiomerically pure standards. The essential oils were analyzed for their sensory profile by gas chromatography-olfactometry (GCO) and evaluated by means of a dilution analysis technique (AEDA).

Results / Discussion / Conclusion

In *M. myrsinoides*, sesquiterpene hydrocarbons were the main constituents, with (Z)-caryophyllene (16.8%), trans-calamenene (14.6%), 1.8-cineole (11.6%), spathulenol (6.5%) and limonene (5.2%) as major compounds. In the same specie we determined eight enantiomeric pairs and eight active-olfactory compounds, of which limonene present the highest dilution factor (FD). For what concerns the species *M. mollis*, monoterpene hydrocarbons were the main constituents. β -pinene (31.3%), α -pinene (29.2%), 1.8 - cineole (8.7%), linalool (8.2%) and myrcene (5.2%) were the major compounds. Five enantiomeric pairs were identified and the compounds with the highest FD were β -pinene and 1.8-cineole. With respect to the inhibitory activity, the essential oil of *M. myrsinoides* was active against acetylcholinesterase AChE and butyrylcholinesterase BChE with an IC₅₀ of 78.6 and 18.4 $\mu\text{g} / \text{ml}$, respectively. These values are very close to those determined by Bonesi et al., 2010 for the essential oil of *P. heldreichii subsp. Leucodermis*, whose IC₅₀ was 51.1 $\mu\text{g} / \text{ml}$ anti-AChE and 80.6 $\mu\text{g} / \text{ml}$ anti-BChE.

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Curcumin prevents oxidative damage in mouse embryos exposed to cadmium

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Introduction

Cadmium is used as an experimental model for the induction of neural tube defects; one of its main proposed teratogenic mechanisms is oxidative damage. Curcumin (CUR) or diferuloylmethane, an active compound of the rhizome of the *Curcuma longa* plant, presents a broad spectrum of pharmacological activities (1). As an antioxidant, it protects against oxidative damage by acting as a scavenger of free radicals and inhibiting ROS generation both *in vivo* and *in vitro* (2). In addition, the possible metal-ligand interaction of Cd-CUR has been suggested, which would contribute to its pharmacological activity (3). Thus, the objective of this work was to evaluate the antioxidant effect of CUR both *in vivo* using embryos from pregnant mice exposed to cadmium, and *in vitro*, in addition to evaluating its chelating activity.

Method

The levels of lipid peroxidation by TBAR's method were determined in ten-day-old embryos from animals to which 100 mg / kg of curcumin weight was administered per day, from the gestational day zero to the tenth day. Cadmium was administered via IP (1.75 mg / kg of CdCl₂) on day 7 of gestation. In order to a better understanding of the protective effect of curcumin, an *in vitro* assay was carried out to determine its antioxidant activity, determining its capacity to scavenge the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Finally, its possible chelating activity was determined by proton nuclear magnetic resonance.

Results / Discussion / Conclusion

Concerning the antioxidant activity, curcumin (100 mg / kg) prevented the increase in lipid peroxidation produced by cadmium in exposed embryos. Likewise, it showed to have a high antioxidant activity *in vitro*, surpassing the reference used for this test (ascorbic acid), since it showed a higher capacity to trap free radicals at very low concentrations, to which the ascorbic acid did not show stabilizing activity for the DPPH. Vitamin C has 2 binding sites to the DPPH radical, the hydroxyl groups; the CUR has a greater number of hydroxyls so it could stabilize more DPPH molecules; in addition, it has a very conjugated structure, which could lead to more union sites (4). Finally, the results obtained in the 1H NMR spectra, in

agreement with previous reports (3,5), suggest a possible chelating activity; however, it is necessary to extend the studies to be able to assert it. Conclusion: Curcumin presents protection against the lipoperoxidation caused by cadmium in mouse embryos. The protective effect against lipid peroxidation is possibly due to the sum of its activities as antioxidant and probably chelant, as well as its practically null toxicity.

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Curcumin protects from teratogenesis caused by cadmium in mouse

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Introduction

The main exposure to cadmium occurs when breathing tobacco smoke or consuming contaminated food and beverages. The main target organs of the metal are the kidneys, lungs and bones (1). However, it also exhibits teratogenic activity, therefore, in addition to its exposure during human pregnancy represents a risk, it is also used as a model for the induction of neural tube defects in embryos (2). On the other hand, curcumin, the active principle of *Curcuma longa*, has attracted the interest of scientists because it has a broad spectrum of pharmacological activities, including antioxidant, antitumor, anti-inflammatory, antibacterial, antifungal, antiviral, anticancer and anticuagulant (3). Thus, the objective of this work was to evaluate curcumin capacity to prevent teratogenesis caused by cadmium in a murine model.

Method

Pregnant ICR: CD1 mice were used to which curcumin was administered intragastrically from day zero to day 17 of gestation in doses of 25, 50 and 100 mg / kg of animal weight; on day 7 of gestation (7 DG) a dose of 1.75 mg / kg of CdCl₂ was administered intraperitoneally. On day 17 DG, the animals were sacrificed and a hysterectomy was performed to record the number of corpora lutea, number of live and dead fetuses, number of implantations, number of early and late resorptions, weight and size of placentas, fetal weight and length, as well as external malformations. The analysis of the skeletal abnormalities was carried out with one third of each litter using the Peters bichromatic technique. The remaining two thirds were processed to determine visceral malformations by Wilson razor blade slices method.

Results / Discussion / Conclusion

The different doses of curcumin used in this work, did not present a teratogenic effect in mice of the ICR strain. In addition, it protected from the teratogenesis produced by cadmium by considerably reducing the incidence of external, visceral and skeletal malformations in the progeny. The dose that best protected against teratogenesis caused by cadmium was 100 mg / kg. The protection exerted by curcumin by decreasing the number of malformations may be due to the fact that it has the ability to modulate cell adhesion molecules (CAM), its antioxidant activity and its possible chelating activity (4, 5). Concerning skeletal anomalies, cadmium interferes with the formation and maintenance of bones, but curcumin could



counteract this effect by increasing the expression of the *PheX* gene (5). Conclusion: Curcumin protects the teratogenic effect of cadmium in mice. That protection may be due to the sum of its pharmacological activities including antioxidant and chelating, among others. Due to this and to its practically null toxicity, curcumin is suggested as a molecule with potential to prevent teratogenesis caused by agents with mechanisms of action similar to cadmium.

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Preparación de nuevas sulfonamidas derivadas de carbohidratos como potenciales inhibidores de la carbónico anhidrasa

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Introducción

La glucosamina es un aminosúcar que se encuentra de manera natural en el organismo y que actúa como precursor de proteínas y lípidos. También se encuentra en el exoesqueleto de artrópodos, formando la quitina, en la pared celular de los hongos y en otros muchos organismos, así como en algunos alimentos cotidianos.

Las carbónico anhidrasas (CAs) son metaloenzimas expresadas en una amplia gama de organismos pertenecientes tanto a las familias procariotas como a las eucariotas,¹ las CA catalizan una reacción fisiológica que es la hidratación de CO₂ para dar bicarbonato y protón. El sitio activo de la mayoría de las CA contiene un ion de zinc (Zn²⁺), que es esencial para la catálisis.²

La inhibición de las CA tiene aplicaciones farmacológicas en muchos campos, como los diuréticos, agentes antiglaucoma, anticonvulsivos, anticancerígenos,¹ antifúngicos y antibacterianos.³

La principal clase de inhibidores de las CA está constituida por moléculas que contienen el grupo sulfonamido (R-SO₂NH₂); se ha demostrado que la unión de restos glicosídicos a fragmentos que contienen el grupo sulfonamido permite llevar a cabo la inhibición selectiva de las isoformas IX y XII, implicadas en procesos tumorales. La presencia de restos de azúcares también permite incrementar la solubilidad en agua.¹

Método

Para la obtención de estos derivados se siguió la metodología empleada por Fernández-Bolaños et al.⁴ para la preparación de pseudonucleósidos. Se ha partido de los isotiocianatos de sulfonamidas comerciales, que se hicieron reaccionar con d-glucosamina para dar lugar a las tioureas intermedias no detectadas; estos compuestos experimentan, de manera espontánea, el ataque nucleófilo del grupo NH-3 sobre el carbonilo latente. De esta forma se obtuvieron las 5-hidroxi-4-polyhidroxi-imidazolidina-2-iones.

Posteriormente, estos derivados se sometieron a una ciclodeshidratación con AcOH para dar lugar a la formación de las correspondientes glucofuranoimidazolidina-2-tionas bicíclicas.

Resultados / Discusión / Conclusión

Se llevó a cabo la transformación convergente de aminosulfonamidas en pseudonucleósidos como potenciales inhibidores de carbónico anhidrasa. Las etapas clave fueron el acoplamiento de los isotiocianatos de sulfonamidas con hidrócloruro de D-glucosamina natural, seguido de deshidratación catalizada por ácido.

Agradecimientos

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Reduction of bacterial load in wastewater through the use of essential oils

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Introduction

According to the Food and Agriculture Organization of the United Nations wastewater can be defined as a liquid that has no value for immediate use, because it contains a large amount of organic matter, mainly human and animal waste. The International Organization of Standards (ISO) defines essential oils as "Product obtained from vegetable raw materials, either by distillation with water or steam, or from the epicarp of the fruits by mechanical processes or by dry distillation". In the present study, the decrease of total and fecal coliforms in wastewater from the three urban rivers of Loja Ecuador using essential oils was evaluated.

Method

For the choice of the essential oil, four factors were taken into consideration: antimicrobial properties, availability, and yield (essential oil / vegetable material). The essential oils were obtained by hydrodistillation and then analysed by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

The concentrations used of essential oil were between 1 ppm and 100 ppm. The emulsions (O/W) were made mechanically and through the use of an emulsifier. Membrane Filtration Method was used for the determination of total and fecal coliforms.

Results / Discussion / Conclusion

The selected essential oils were lemon (*Citrus aurantifolia*) and eucalyptus (*Eucalyptus globulus*). The major components of lemon oil were limonene (58.0%) and α -terpinene (10.1%) and eucalyptol (77.3%) was the major component in oil of eucalyptus. The concentrations used for lemon were 1, 5, 10 and 100 ppm and for the eucalyptus oil concentrations of 1, 2.5, 5, 8, 10 and 100 ppm were used. With 100 ppm of *Citrus aurantifolia* oil the 95% decrease in total coniforms was achieved. The essential oil of *Eucalyptus globulus* achieved a decrease of more than 95% of fecal and total coliforms with 10 ppm and 100% with 100 ppm. The most stable emulsions for the oil of *Citrus aurantifolia* were those obtained mechanically with 10 ppm of oil. For the oil of *Eucalyptus globulus* the concentrations of 1:1 and 1:0.5 oil:emulsifier were used and the emulsions proved to be stable for all concentrations of essential oil, with creaming speeds lower than -0.0012 m/s.

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Efecto de los extractos acuosos de *Milleria quinqueflora* y *Erythraea tetramera Schiede* sobre la actividad de la lipasa pancreática, adipogénesis y lipólisis en células 3T3-L1.

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Introducción

Las plantas han sido utilizadas por milenios y fueron por mucho tiempo la única medicina en todas las civilizaciones. Países orientales como China reconocen completamente el valor de la medicina tradicional y la incorporan al cuidado de la salud junto con la medicina ortodoxa. En muchos casos las plantas medicinales han sido eficaces de igual manera o mejor que los fármacos para el tratamiento de muchos problemas comunes de salud. Estudios clínicos han demostrado que algunas plantas medicinales ayudan en el control y pérdida de peso, teniendo nulos efectos secundarios, siendo estos, alternativas idóneas para la población. La Cocolmea (*Milleria quinqueflora*) y la Tlanchalagua (*Erythraea tetramera Schiede*) se han utilizado a lo largo de los años como tratamiento coadyuvante en el sobre peso y la obesidad, sin ser estudiadas previamente para determinar su efecto en la pérdida o el control de peso.

Método

Se obtuvieron los extractos acuosos de las plantas en estudio por medio de maceración, filtrado, concentración y liofilizado. En la evaluación de la actividad de la actividad inhibitoria de la lipasa pancreática se utilizó el kit de Spinreact, LIPASA-LQ el cual nos proporciona la concentración catalítica de lipasa al contacto con los extractos acuosos en concentraciones de 5µg/mL y 40µg/mL para *Erythraea tetramera Schiede* y 5µg/mL, 40µg/mL, 50 µg/mL y 400 µg/mL para *Milleria quinqueflora*, utilizando Orlistat como control positivo. Para la identificación de los extractos con efectos sobre los mecanismos relacionados con la captación y acumulación de grasa en los adipocitos se utilizó la línea celular de pre-adipocitos de ratón 3T3-L1 adquirida a la American Type Culture Collection (ATCC) las cuales se

sembraron en placas de 6 pozos siguiendo el método de diferenciación propuesto por Zebisch y cols en 2012 (1). Posteriormente se agregaron los extractos acuosos de las plantas en estudio en concentraciones de 5µg/mL y 40µg/mL para cada extracto. Para el proceso de adipogénesis se agregaron los extractos en el día 3 de diferenciación, mientras que para el proceso de lipólisis se agregaron los extractos en el día 11. Al finalizar el proceso de diferenciación se procedió a la tinción por medio de Oil Red O de acuerdo al método descrito por Ramírez-Zacarías y cols (2).

Resultados / Discusión / Conclusión

En el ensayo para la inhibición de la lipasa pancreática, el extracto de *Erythraea tetramera Schiede* presentó una inhibición de 18.7% y 49.9% en las concentraciones de 5µg/mL y 40µg/mL respectivamente, mientras que *Millieria quinqueflora* presentó 14.1%, 21.74%, 120.52% y 112.3% para las concentraciones de 5µg/mL, 40µg/mL, 50 µg/mL y 400 µg/mL respectivamente respecto al control positivo Orlistat que presentó 60%, efecto similar al que describen Mendoza Mesa y Medina Valdés (2014) (3) atribuyendo la capacidad inhibitoria del extracto a la cantidad de polifenoles presentes en los mismos. Los extractos de *Erythraea tetramera Schiede* y *Millieria quinqueflora* inhibieron la adipogénesis en concentraciones de 5µg/mL y 40µg/mL adicionados al día 3 del proceso de diferenciación, en las células 3T3-L1, por otro lado inducen la lipólisis en las células 3T3-L1 al agregar los extractos en concentraciones de 5µg/mL y 40µg/mL en el día 11 de diferenciación.

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Elicitation of isoflavonoid compounds in mung bean (*Vigna radiate* L.) sprouts

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Introduction

Mung bean (*Vigna radiata* L., Fabaceae) sprouts, called also “Chinese roots”, constitute one of the most important legumes in the human diet worldwide, due to their high nutritional and medicinal values. Mung bean sprouts are a good source of dietary protein, carbohydrates, minerals and vitamins. Recently, there is a growing interest in the application of elicitors in plants to increase the levels of bioactive substances, such as phenolic compounds and isoflavonoids. Consequently, the use of elicitors could improve the nutritional content and antioxidant capacity. Isoflavonoid content of mung bean includes isoflavones (genistein and daidzein), coumestrol (a coumestan), and the pterocarpans, phaseollin and phaseollidin. Isoflavones and coumestrol are known for their antioxidant and anti-inflammatory properties. In addition to the beneficial effects for human health, these compounds also constitute chemical defense substances of the plant against fungal diseases. The aim of the present research was to evaluate the elicitor effect of the compounds salicylic acid, acetylsalicylic acid, methyl jasmonate and indanoyl L-isoleucine methyl ester on the content of genistein, daidzein, coumestrol, phaseollin and phaseollidin in mung bean sprouts.

Method

The sprouts of mung bean were purchased in a local market. Sprouts were immersed in solutions of each elicitor (at different concentrations) for 4 hours; then, vegetal material was stored during 24, 48, 72 and 96 h. Sprouts treated with distilled water were used as negative control. For the analysis, 5 grams of plant material were used and extracted by maceration with ethanol. After filtration, the solvent was evaporated and the residue extracted with ethyl acetate. Once again the solvent was evaporated and the extract was re-dissolved in methanol. The content of isoflavones in the extract was determined by HPLC using calibration curves. It was evaluated the post-induction time as well as the elicitor dose-response effect.

Results

Treatments with all elicitors showed a remarkable enhancement in the concentration of isoflavones, coumestrol and the pterocarpans, phaseollin and phaseollidin. The composition depended on the structure and dose of the elicitor and the post-induction time. Salicylic acid and acetylsalicylic acid showed phytotoxic effects in the mung bean sprouts and, after 72 hours, amount of isoflavones decreased.

Conclusion

Treatment with elicitors can be used to increase the levels of bioactive compounds such as isoflavones, coumestrol and phaseollin in mung bean sprouts. Structure and doses of elicitor, along with post-induction time, may be taking into account to avoid phytotoxic effects.

Amphotericin B topical cream using essential oil of *Bursera graveolens* for the treatment of candidiasis

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Introduction

Cutaneous fungal infections produced by various species of *Candida* cause the pathogenesis known as candidiasis. Some drugs including fluconazole, ketoconazole, itraconazole, clotrimazole, among others are used for the treatment of mycoses. However, resistance mechanisms to these antifungals have been detected [1]. Amphotericin B (AmB) is a broad-spectrum polyene macrolide with high activity against the most common fungi that cause cutaneous mycoses. It is considered effective against azole-resistant fungus. AmB is an amphoteric and amphiphilic molecule that is highly insoluble in water, which are properties that block an optimal cutaneous permeation [2]. Essential oil of *Bursera graveolens* (BG-oil) is rich in terpenes such as limonene and therefore could be used as a permeation enhancer of AmB. Moreover, antifungal activity of this oil has been previously reported [3]. Considering that there are no topical formulations of AmB commercially available, the aim of this study was to design an AmB topical cream using BG-oil in order to improve the permeation and efficacy of AmB.

Method

Compositions of AmB-cream with and without BG-oil were established by a 2³ factorial design. Physicochemical characterization included viscosity, rheological behavior, morphological characterization and extensibility. *Ex vivo* permeation using human skin as well as antifungal efficacy against strains of *Candida albicans* were determined. Finally, the tolerability was determined by evaluating biomechanical properties of skin from human volunteers.

Results / Discussion / Conclusion

Two homogeneous pharmaceutical creams were obtained: one containing BG-oil (AmB/BG-cream) and the other without BG-oil (AmB-cream). Both formulations presented similar physical properties such as droplets



of spherical shape and uniform size, pseudoplastic behavior, and good spreadability with a hyperbolic profile. The viscosity was 884.6 ± 33.28 mPa·s for AmB/BG-cream and 939 ± 33.07 mPa·s for AmB-cream. *Ex vivo* permeation studies revealed that AmB of both formulations did not permeate through human skin. However, it was observed that AmB was highly retained in the skin with values of $982.2 \mu\text{g}/\text{g}/\text{cm}^2$ for AmB/BG-cream and $721.4 \mu\text{g}/\text{g}/\text{cm}^2$ for AmB-cream, which suggests that BG-oil facilitates the diffusion ability of the drug through the stratum corneum thus increasing its concentration in the skin layers in order to provide a local effect. The antifungal action of AmB against *Candida albicans* was clearly observed in both formulations with AmB/BG-cream having a lower minimal inhibitory concentration (MIC) thereby making it more effective than AmB-cream, which suggests that BG-oil potentiates the pharmacological efficacy of the formulation. The tolerance study showed that both formulations do not cause destabilization nor damage to the skin surface of volunteers. Consequently, these results suggest that AmB/BG-cream could be used as a local action treatment for cutaneous mycoses, including candidiasis.

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Evaluación de la capacidad biodegradadora de *Penicillium sp.* y *Aspergillus flavus* en polietileno tereftalato

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Palabras clave: Polietileno tereftalato, *Penicillium sp.*, *Aspergillus flavus*, Biodegradación

Resmen.

La biodegradación del polietileno por medio de microorganismos en la actualidad es una solución para la reducción de la contaminación por plásticos. En la presente investigación, se obtuvo el aislamiento y caracterización de dos cepas de hongos *Penicillium sp.* y *Aspergillus flavus* capaces de degradar el polietileno. **Metodología**, se obtuvieron muestras de PET de diferente calibre del No.3, 6 y 11; las cepas se aislaron y se aplicaron en medios de cultivo sólido y líquido con y sin sacarosa, respectivamente, con la finalidad de observar el crecimiento micelial en las diferentes muestras de PET y permitió evaluar la actividad biodegradadora basándonos en la tabla para tal fin (Méndez *et al.* 2007. *El objetivo* de esta investigación es la evaluación de las cepas *Penicillium sp.* y *Aspergillus flavus* para determinar la cepa con mayor eficiencia en la desintegración del PET. **Resultados:** La cepa de *Aspergillus flavus* resulto ser mas eficiente para la biodegradación para los tres tipos de PET (3,6 y 11).

Palabras claves: Polietileno tereftalato, *Penicillium sp.*, *Aspergillus flavus*, biodegradación.

Introducción

El plástico se puede incorporar a cualquier proceso productivo o bien, a un producto final, razón por la que es innegable que los productos plásticos tienen un lugar sobresaliente en nuestra economía.¹ El Estado de Guerrero no ha sido la excepción, pues hoy en día se generan 3 mil toneladas de plásticos al día que no son recicladas de manera correcta.^{1,2}

Método

La actividad biodegradadora se midió a través del crecimiento micelial en placas de PET de calibres No.3, 6 y 11 expuestas con un medio de cultivo sin sacarosa en un periodo total de 6 meses divididos por ciclos de 1 mes y medio, 3 meses y 6 meses. Los resultados obtenidos fueron corroborados mediante micrografías que

se hicieron en un microscopio electrónico de barrido JEOL IT-300 LV con un espectrómetro de dispersión de energía de rayos x (EDS) BRUKER XFLASH 6/30.

Resultados / Discusión / Conclusión

La cepa de *Aspergillus flavus* tuvo un crecimiento micelial favorable a diferencia de la cepa de *Penicillium sp.* en el calibre No.3, dicho crecimiento es notorio a partir de los 3 meses *Penicillium sp.* y *Aspergillus flavus* poseen capacidad degradadora. *Aspergillus flavus* es superior que *Penicillium sp.* ya que logró degradar un porcentaje mayor. Con esta investigación se logra comprobar que existen diferentes alternativas como la utilización de hongos microscópicos para erradicar un poco el problema del exceso de plásticos en el mundo.

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Al Laboratorio de Microscopía Electrónica de Barrido y Microanálisis (LMEByM) a cargo del Dr. Oscar Talavera Mendoza.

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Evaluación de la permeación cutánea de un sistema de liberación prolongada conformado por ibuprofeno y aceites naturales

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Introducción

El ibuprofeno es un fármaco antiinflamatorio no esteroideos, ampliamente usado para el tratamiento de artritis reumatoide, osteoartritis, pero presenta baja solubilidad lo que limita su permeabilidad. Por otra parte, el aceite de oliva extra virgen, que pertenece a la familia *Oleaceae*, también muestra propiedades antiinflamatorias por la presencia de un compuesto fenólico denominado descarboxi aglicona metil ligustrósido (oleocantal). El aceite de ortiga (*Urtica dioica*), es otra sustancia de origen natural que por la presencia en su composición del kaempferol se le atribuyen propiedades terapéuticas: antioxidantes, antimicrobianos, antiinflamatorias, analgésica, entre otras.

Los cristales líquidos (CL) son sistemas con estabilidad termodinámica, muy estudiados debido a su afinidad con moléculas lipofílicas e hidrofílicas. Adicionalmente proporciona considerables ventajas, tales como: capacidad de aumentar la biodisponibilidad de moléculas activas, direccionar fármacos a sitio diana, proteger contra la degradación química, y por su naturaleza anfifílica también pueden cruzar las capas de la piel y facilitar la permeación de principios activos hacia la sangre. En vista de todo lo mencionado previamente, en el presente estudio se evalúa la permeación cutánea de un sistema de liberación prolongada conformado por ibuprofeno y aceites naturales

Métodos

La extracción del aceite de ortiga fue realizada empleando el método Soxhlet y caracterizado por cromatografía de gas, el aceite de oliva se obtuvo de forma comercial. Los CLs fueron preparados con la construcción de dos diagramas de fases, uno para cada aceite estudiado, se combinó diferentes concentraciones de agua, aceite (*Olea europea* o *Urtica dioica*) y tensoactivo (cooperlam). Para el estudio de solubilidad del ibuprofeno se procedió en adicionar sucesivas dosis de 0,01g de fármaco hasta alcanzar su saturación, las muestras fueron caracterizadas por microscopia de luz polarizada. La permeación y retención percutánea *in vitro* fue realizado por un periodo de 48 horas utilizando piel de oreja de porcino. Al final del

análisis, se removió la piel de la célula de difusión y se realizó la técnica *tape stripping*. Todas las muestras fueron cuantificadas por espectroscopia UV. Posteriormente con la muestra que presentó el mejor perfil de permeación, se desarrolló una forma farmacéutica semisólida (crema) de aplicación tópica. Para facilitar la interpretación de los sistemas, se consideró las siguientes siglas: CL-O, CL-U, CL-OI, CL-UI, AO y AU para los cristales líquidos con aceite de oliva, cristales líquidos con aceite de *Urtiga dioica*, cristales líquidos aceite de oliva/ibuprofeno, cristales líquidos *Urtiga dioica*/ibuprofeno, aceite de oliva y aceite de *Urtiga dioica* respectivamente.

Resultados / Discusión / Conclusión

Se encontró que el componente mayoritario del *Urtiga dioica* fue el ácido linoleico, el diagrama de fases para ambos sistemas (CL-O y CL-U) reveló la formación de sistemas viscosos y transparentes en distintas regiones. Fue posible incorporar 200mg de fármaco a cada uno de ellos. Todos los sistemas mostraron presencia de estrías, lo que indica la formación de cristales líquidos de fase hexagonal. Ambos aceites aumentaron la permeación percutánea del ibuprofeno, sin embargo, la actividad del AO fue superior al AU comparados con ibuprofeno libre en 24 horas. Los CL-O y CL-U presentaron 40% de ibuprofeno permeado en 24 horas, pero en este período, los sistemas (CL-O) mostraron un descenso para aproximadamente 20% de fármaco liberado, frente a 32% para los CL-U en las 48 horas. Por tanto se considera que el ibuprofeno presenta más afinidad por el AU cuando es incorporado al sistema de liberación. En este sentido, se preparó la crema con los CL-U. Las microscopias mostraron la formación de estrías para la crema con CL-U, lo que sugiere que la forma farmacéutica no cambió la estructura del sistema. Con lo expuesto, se sugiere que los cristales líquidos con la composición estudiada son sistemas prometedores en la permeación de ibuprofeno.

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Homoisoflavonoide aislado de la madera de *Caesalpinia bahamensis*. Caracterización espectroscópica y actividad antibacteriana

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Introducción

Las plantas medicinales han probado históricamente su valor como fuente de moléculas con potencial terapéutico y actualmente representan uno de los precursores más importantes para la identificación de novedosas drogas¹.

La emergencia de la resistencia bacteriana ha generado nuevos intereses en la búsqueda de medicamentos con poder antibacteriano, prueba de ello es el aumento en los últimos años del número de publicaciones, relacionando productos naturales y actividad antimicrobiana, centrándose las investigaciones en los productos naturales como fuentes de moléculas bioactivas².

Caesalpinia bahamensis subsp. *orientensis* Borhidi es una especie perteneciente al género *Caesalpinia* de la familia *Fabaceae*. *C. bahamensis* subsp. *orientensis* se localiza fundamentalmente en la costa norte del archipiélago de Cuba, así como en las Bahamas³. Es empleada en la medicina herbolaria popular para el tratamiento de infecciones de los riñones, que pudieran estar asociadas a infecciones bacterianas. Se conoce muy poco acerca de la fitoquímica de esta planta, así como de su actividad antibacteriana.

Métodos

La madera seca y desgrasada con n-hexano, se extrajo con etanol por maceración asistida con un baño ultrasónico. Posteriormente, el crudo etanólico suspendido en una disolución hidroalcohólica (1:1), fue extraído con cloroformo y acetato de etilo. Se evaluó la actividad antibacteriana de los extractos frente a cepas bacterianas de interés farmacológico empleando el método de microdilución y se obtuvieron los valores de concentración mínima bactericida. El extracto clorofórmico fue fraccionado mediante cromatografía de adsorción, y a partir de una las fracciones reunidas se aisló el metabolito mayoritario mediante cromatografía preparativa de capa delgada. La caracterización del metabolito mayoritario se realizó

a través de experimentos de RMN mono y bidimensionales, así como espectrometría de masas de alta resolución. Igualmente, fue evaluada la actividad antibacteriana del compuesto aislado empleando el método de microdilución.

Conclusiones

La evaluación de la actividad antibacteriana de los extractos de la madera de *C. bahamensis* frente a cepas de *S. aureus* y *E. coli* mostró que los mismos poseen una buena actividad antibacteriana frente a las cepas ensayadas. La caracterización espectroscópica del metabolito mayoritario de la madera de *C. bahamensis* permitió identificar la estructura correspondiente al (6aS,12S)-7,11b-dihidroindeno[2,1]-cromeno-3,6a,9,10(6H)-tetraol (brazilina), un homoisoflavonoide que se reporta por primera vez para la especie, si bien ha sido aislado de otras especies del mismo género. La caracterización por espectrometría de masas de alta resolución evidenció la baja estabilidad de este homoisoflavonoide al estar expuesto a la luz, pues su ion molecular desprotonado correspondió a la brazileína, que es su producto de oxidación. La brazilina solo mostró actividad bactericida frente a *S. aureus*, con un valor de CMB = 12,5 mg/mL, que resultó menor que la del extracto del que fue aislado, lo que pudiera estar asociado al efecto sinérgico del resto de los componentes de la fracción y/o extracto. Se reporta por primera vez la evaluación de la actividad antibacteriana de los extractos de esta planta.

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³Roig, J.T., **1988**

Evaluación de la actividad ansiolítica de *Erythrina americana* Mill. (Colorín)

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Introducción

Erythrina americana Mill. es una especie endémica mexicana utilizada en la medicina tradicional por sus efectos tranquilizantes (Hastings, 1990; Garín-Aguilar *et al*, 2000). El objetivo de este trabajo fue evaluar el efecto ansiolítico del extracto metanólico de semillas de *E. americana* (EAM).

Método

El extracto metanólico de las semillas previamente desengrasadas, se obtuvo por extracción con metanol en Soxhlet. Posteriormente, se realizó el análisis preliminar fitoquímico (APFQ) y una cromatografía líquida de alta eficacia (HPLC) para la identificación de ácidos fenólicos, flavonoides, terpenoides y alcaloides. En todas las pruebas farmacológicas se emplearon ratones macho ICR (CD-1). La toxicidad aguda del EAM (10-5000 mg/kg) se estimó siguiendo el método descrito por Lorke (1983). El efecto ansiolítico se evaluó usando el modelo de Laberinto en Cruz Elevado (Pellow *et al.*, 1985). Los animales se distribuyeron al azar en grupos independientes (n=9) para recibir EAM (10, 20, 40, 60 ó 80 mg/kg i.g.), Diazepam (1 mg/kg i.p.), vehículo (NaCl 0.9 % i.g.) y se incluyó un grupo sin tratamiento (INT). Para descartar el posible deterioro motor ocasionado por los tratamientos, se evaluó la actividad motora de los sujetos en el modelo de Campo Abierto (Hall, 1934).

Resultados / Discusión / Conclusión

El APFQ detectó la presencia de alcaloides, flavonoides, saponinas, taninos, quinonas, azúcares reductores y glicósidos cardiacos y el HPLC identificó ácidos fenólicos: gálico, clorogénico, vainillínico, p-hidroxibenzoico, cafeico y p-cumárico; flavonoides: apigenina, floretina, florizidina, miricetina y naringenina; terpenoides: ácido ursólico, carnosol, stigmasterol, α -amirina y β -sitosterol; y alcaloides: erisodina, β -eritroidina, erisopina y erisovina. El EAM no resultó tóxico aun en dosis de 5 000 mg/kg. Las dosis 40 y 60 mg/kg del EAM tuvieron efecto ansiolítico semejante al Diazepam sin deteriorar la actividad motora horizontal de los animales. El efecto ansiolítico observado, puede estar mediado por la interacción química de los componentes identificados en el EAM. Este estudio evidenció el potencial de *Erythrina*

americana como fuente de agentes ansiolíticos y proporciona un soporte científico del uso empírico de esta planta como tratamiento efectivo en padecimientos relacionados con la ansiedad. Este es el primer reporte de actividad ansiolítica con *E. americana*.

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Antimicrobial activity of poison obtained from freshwater stingray *Potamotrygon magdalenae*

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Introduction

The freshwater rays belong to the order Myliobatiformes (rays), to the family Potamotrygonidae (Garman, 1877), represented by 3 genera: *Paratrygon*, *Potamotrygon*, *Plesiotrygon*. The genus *Potamotrygon* has the highest number of species and, therefore, has a large specific and individual variation. In this particular genus, it is characterized by the presence of a poisonous sting. The stinging epithelium of the *Potamotrygon magdalenae* species was studied, from the bioprospecting approach of molecules of biotechnological interest that are produced by this sweet species in Colombia. The investigation includes the analysis of the potential of the toxins extracted from the stripes and for this, the individuals were collected the epithelia of individuals of both sexes and of the different stages of the species. The crude extracts of the venom collected were analyzed to determine their antimicrobial potential, in different in vitro tests, in microorganisms that cause nosocomial infections, of which significant percentages (up to 100%) were obtained with values of minimum inhibitory concentration (MIC). (for *Candida albicans* = 0.08706, R2 = 1.0, *Pseudomonas aeruginosa* = 0.1807, R2 = 1.0, *Klebsiella pneumoniae* = 0.001, R2 = 1.0). These results allow us to infer that these species have an antimicrobial potential, which can result in obtaining promising molecules with antifungal and antibacterial activity.

Methods

To obtain the venom, the sting epithelium was removed and rinsed in buffered saline (phosphate buffer pH 7.0), then transported on dry ice to the laboratory, where it was stored at -20 ° C until use. Tests against bacteria and fungi were carried out in LB liquid medium (50% bactotrypton + 25% yeast extract + 35% NaCl, diluted in distilled water) and Sabouraud, respectively; using negative control (the medium) and positive control (gentamicin 40 µg.mL⁻¹ for bacteria and clotrimazole 10 µg.mL⁻¹ for yeasts). Growth was determined by spectrophotometry at consecutive time readings using a wavelength of 600 nm, monitored by the ELISA reader, every 30 minutes of shaking the 96-well plate in a shaker at 37 °C and 50 RPM. Bacteria strains obtained from the American Type Culture Collection (ATCC), Gram positive: *Staphylococcus aureus* (ATCC 13709) and, *Enterococcus faecalis* (ATCC 14428) and Gram negative as *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC 11229), as well as yeasts of the genus *Candida sp.* were used.

Results / Discussion / Conclusion

The results show that extracts of the older specimens (regardless of sex) can inhibit more than 50% yeast *Candida albicans*; and young specimens can inhibit close to 40% in the log phase of growth. In the assay against *Pseudomonas aeruginosa*, a behavior was found, but tending to a straight line where the extracts did not show a difference in the inhibition of the extracts obtained from the males, whereas the extracts of the females were able to inhibit the bacterial growth. For the *Escherichia coli* assays, there is no pattern for the same sex extracts, nor the age group, since the inhibitory effect was for immature females and males of



different magnitude. The bioassay performed for *Klebsiella pneumoniae* had the best results on the behavior of the extracts, both in inhibition as well as in the permanence of this inhibition in the extracts and the dilutions. Dilutions up to 1×10^{-2} of the extract inhibit at least 80% bacterial growth. In the case of *Staphylococcus aureus*, the growth behavior against all extracts, showed that the larger dilutions (up to 1×10^{-2}) have the inhibitory effect. For specific MICs, it is very interesting that the effect is achieved at a low concentration of $0.0131 \mu\text{g}\cdot\text{mL}^{-1}$ for *C. albicans* and even lower for *K. pneumoniae*. In the bioassays with the crude extracts against *K. pneumoniae* and the inhibitory effect of 100% and the significance of the test, still at the maximum dilution used (1×10^{-4}), is an important result of antibacterial activity. same against yeasts. *C. albicans*, which were significantly inhibited at the lowest dilution (1×10^{-3}). Significance in comparisons shows that the results can be very promising in order to identify molecules from poison useful to be used at the future in new biotechnological products.

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POSTER

The compound 8pp alters the cellular organization of azole-resistant *Candida albicans*

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Introduction

We had previously demonstrated the antifungal activity of the flavonoid 2', 4'-dihydroxy-5' - (1'', 1'' - dimethylalil) -8-prenylpinocembrin (8pp), on multi-resistant microorganisms of nosocomial origin (Pérez *et al.*, 2003). The compound 8pp inhibits mitochondrial enzyme activity, proton potential and ATP synthesis in rat hepatocytes.

In addition, it decouples oxidative phosphorylation and produces toxicity in the human cancer cells HEp-2 (Elingold *et al.*, 2008). In azole-resistant *Candida albicans* (RCa), 8pp reverses resistance to azoles, decreases both cell growth and viability *per se* and also cooperates with fluconazole in these phenomena. Concomitantly, it inhibits the azole active transport by interacting with the sites of ABC cdr efflux pumps (Peralta *et al.*, 2012; Barceló *et al.*, 2017).

Objective

To study the effects of 8pp and fluconazole on the cellular permeability of RCa.

Materials and methods

Microorganism: azole-resistant *Candida albicans* of oral origin (RCa, USA). Propidium iodide (PI) cell uptake and observation of morphology by fluorescence microscopy (Shreaz *et al.*, 2016).

Results

The yeasts, collected in the late exponential phase and pre-incubated with 8pp or fluconazole at inhibitory concentrations of cell growth, stained red in their entirety. This indicates damage to its membrane and cell wall, with the subsequent PI uptake and nucleic acid staining. Ethanol, taken as a reference compound, produced an even greater effect.

Conclusions

8pp and fluconazole alter the membrane and cell wall structures of azole-resistant *Candida albicans*, with the subsequent cell death.

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The effect of *Spilanthes oleraceae* Var *oleraceae* leaf extracts on rat induced diabetic.

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Introduction

The *Spilanthes oleracea* L., popularly known in Brazil as Jambu or Agrião-do-Para, is considered a domestic vegetable used as food and cosmetic¹. It is used as an anesthetic, anticonvulsant, analgesic, antimicrobial agent, among others uses². In diabetic patients it acts as a diuretic agent. The objective of the present study was to evaluate the potential of Jambu extract as a glycemic controller in a model of diabetes mellitus.

Material and Methods

A total of 90 g of Jambu leaves, obtained from 60 days old growing plants, were crushed with 200mL of sterile distilled water. After 30 minutes in refrigerator, submitted to lint filtration and quantified total protein and phenol concentration. Male Wistar rats (250 to 280 g) were obtained from UNINOVE animal facility with the approval of the animal ethical committee (process 20/2015). Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (50 mg/kg) in citrate buffer. The experiment involved four groups of rat; Control (non-diabetic rats); DM (diabetic rats); Control+Extract (1mL leaf extract/200g rat); and DM+Extract (diabetic group treated with 1mL of leaf extract). Blood glucose levels were measured 48 hours after the STZ injection, and animals were considered diabetic when blood glucose levels were, at least, 500mg/dL. The groups were evaluated for 30 days, the animals were euthanized, and blood was collected for measurement of glucose. Blood serum was precipitated with ammonium sulfate, followed by dialysis and submitted to HPLC for amino acids analysis. Pancreas was collected for histological analysis.

Results/Discussion/Conclusion

Aqueous extract presented 653.66 mg of protein and 568.60 mg of phenol. Control animals treated with the extract retained the glycemic rate varying from 89 to 94mg/dL, DM rats presented severe hyperglycemia (473 to 524 mg/dL), and with Jambu extract showed a reduced average glucose serum value (201mg/dL). Plasma from control and treated rats presented Tyr and Met, however, these amino acids were undetectable in diabetic's rats, being that in these was detected kynurenine (Kyn) in plasma. Treatment with Jambu extract reestablished the Try and Met levels, decreasing Kyn. Analyses of the pancreatic tissue showed a loss in pancreatic islet total area in diabetic rats and Jambu extract reestablished it. Treatment involved beta-cell

regulation, therefore controlling blood glucose levels.. In conclusion aqueous extract of *S. oleraceae* ameliorates glyceimic exacerbation in the STZ-model, pointing to an alternative treatment for diabetes mellitus. Acknowledgments: Financial Support from CNPq 474681 / 2013-0.

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Supplementation of the flour cashew apple in cashew apple juice increases immune response

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Introduction

Anacardium occidentale Linn (cashew) belongs the Anacardiaceae family, is original from Brazil and especially cultivated in the Northern and Northeastern regions for industrial purpose. The peduncle, also known as cashew apple, is juicy, has a pleasant flavor and it is preferably marketed as frozen pulp, juice, and nectar. The cashew apple bagasse is rich in insoluble fibers (dietary fiber content corresponding to 60% of dry weight), nutrients, and bioactive compounds (Fontenele et al., 2017). The annual cashew processing is mainly destined to obtain cashew nuts. For each ton of cashew nuts, 10 to 15 tons of cashew apples are produced as by-products, 10 from which 65–80% of juice is recovered, resulting in 2 to 4 tons of cashew apple bagasse (Das & Arora, 2017). Then, we propose that bagasse flour added to cashew apple juice (CAJ) could be an alternative for the improvement of its nutritional quality. The aim of this study was investigated the effect of the supplementation of the bagasse flour cashew apple in CAJ on immune response in mice.

Method

The bagasse flour of cashew apple was obtained in different stages of maturity, ripe (RF) and unripe (UNF). Therefore, the antioxidant capacity of ripe cashew apple juice and ripe and unripe bagasse flour was evaluated by two methods, ABTS and DPPH (Rufino, 2007). Additionally, polyphenols, vitamin C, carotenoids, anthocyanins and yellow flavonoids were quantified. The Ethics Committee on Animal Research of UFC approved in vivo tests with male swiss mice under the protocol N^o 102/2011. Mice received daily, oral doses (19 days) of CAJ, cashew apple juice added to 75 and 300 mg/kg of ripe cashew apple flour (RFCAJ75 and RFCAJ300, respectively) and cashew apple juice added to 75 and 300 mg/kg unripe cashew apple flour (UNFCAJ75 and UNFCAJ300, respectively). Physiological parameters such as body weight, relative organ to body weight, liver lipid peroxidation, ALT and AST transaminase enzymes activities were determined in sub-acute toxicity test. The immune response was evaluated by haemagglutinating antibody titre (HA) and delayed-type hypersensitivity (DTH) response, using sheep red blood cells (SRBCs) as antigen (Sharma et al., 1996).

Results / Discussion / Conclusion

Total antioxidant capacity by ABTS and DPPH methods indicated that unripe cashew apple flour (UNF) as the greatest in relation to ripe cashew apple flour (RF) and CAJ. Phytochemical profile indicated the polyphenols as the main constituent. During the 19-day experiment, no death, toxic signs and negative symptoms were observed in any animal. CAJ and UNFCAJ (300 mg/kg) showed high levels secondary HA ($p < 0.05$) in relation to control. All treatments, CAJ, UNFCAJ (300 mg/kg) and RFCAJ (300 mg/kg) induced increase at DTH in relation to control ($p < 0.05$). The bagasse flour in cashew apple juice presents immunostimulation activities and can be used as an alternative for nutritional improving to the immune system.

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Ethnobotanic survey and preliminary phytochemical evaluation of medicinal plants used in the communities of Acopiara-CE

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Introduction

Medicinal plants have been present in human life since ancient civilizations. They are extremely important for treatment of diseases being a source of new biologically active components. The purpose of the present work was to investigate ethnobotanical data collection and phytochemical profile of medicinal plants used in a rural and an urban community of Acopiara-CE city.

Method

The study was conducted from March 2015 to February 2016. The ethnobotanical research was based on the application of 50 semi-structured questionnaires for both communities. The preliminary phytochemical evaluation was carried out with the collection of material from *Spondias purpurea* L., *Licania rigid* Benth, *Prosopis juliflora*, *Spondias tuberosa* Arruda and *Caesalpinia pyramidalis* Tul. The stem bark, branches and leaves of the studied plants were shaded for 7 days at room temperature, and then weighed, crushed and extracted using the ethanol maceration method.

Results/Discussion/Conclusion

In ethnobotanical research, 100% of rural community claimed to use medicinal plants while only 67% of the urban community make this traditional use. A total of 40 medicinal plant species used as infusions were cited by both communities. The main therapeutic indications of medicinal plants used by both communities are: headache, flu and fever, diarrhea, sore throat and body pain, among others. This traditional use of medicinal plants was associated to the low cost since the infused tea or plant is cheaper than the health treatment by medicine. In the phytochemical evaluation, tannins, flavonoids, steroids, triterpenoids, saponins and alkaloids were found in almost all species and in different parts of the plants. The *Spondias tuberosa* Arruda has the highest amount of constituents present, being only negative for alkaloids. The highest amount of total tannin (0.93% cyanidin) was obtained in the stem bark of *Spondias purpurea* L after tannin quantification. These results may promote the isolation, purification and structural characterization of compounds for further biological testing in order to characterize their use as a new herbal medicine.

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Green banana biomass in the menu for health promotion at school

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Introduction

Green banana biomass (GBB) is derived from cooked and processed green banana. It has no characteristic flavor and can be added in the preparation of various products, adding nutritional value and functional characteristics of food fibers. The fermentative capacity of fibers is the main characteristic that makes them one of the primary foods for maintaining body health, causing the strengthening of the immune system beside directly influencing disease reduction and prevention (Marques et al., 2017). The National School Feeding Program in Brazil (PNAE) plays an important role in learning, preparing healthy eating habits, and activity in promoting student health. Incorporating GBB into a regular diet for children results in a more nutritious diet. However, the incentive to use green banana pulp/biomass in some food products in school is still scarce. The aim of study was analyzed the acceptance of school meals after the addition of green banana biomass in the menu preparations.

Method

The acceptability test was conducted with 322 students aged 6 to 14 years, from two schools from Redenção city in Ceará, Brazil. To evaluate the acceptability of the preparations, the 5-point facial hedonic scale test was used, which is distributed in “I hated”, “I didn't like”, “indifferent”, “I liked” and “I loved it”. According to the National Education Development Fund (FNDE), the percentage of acceptance of the evaluated preparation should be greater than or equal to 85% including the answer “I liked” and “I loved”. This acceptance value (85%) for each preparation tested demonstrates that the sample was well accepted by the students and allows it to be inserted in the school lunch menu. Two preparations were chosen for the addition of green banana biomass: meat soup and rice with chicken cream. After the green banana biomass preparation they were added to the menu. In the soup, rice and chicken cream were added respectively 15%, 15.14% 6.05% of GBB following the food preparation technical roadmap.

Results/Discussion/Conclusion

The preparations ground meat soup and rice with chicken cream had acceptance of 81% and 89% respectively. The ground meat soup had the following assessment by students: 53% loved it, 28% liked it, 8% were indifferent, 4% disliked it and 7% hated it. The ground meat soup with green banana biomass was evaluated in the morning which is a factor that explains the lower acceptability (<85%) than expected for this preparation. According to the lunch cooks the younger children which study in the morning prefer snack-type preparations (vitamin, fruits, bread, juice) or uncooked foods due their eating habits. The preparation of rice with chicken cream was evaluated by the students as follows: 47% loved it, 42% liked it, 3% were indifferent, 6% disliked it and 2% disliked it. This preparation obtained a satisfactory result of acceptability

(>85%). In the second school these preparations were offers in the afternoon shift. In this period, it is important to offer a menu add with GBB because had the better adherence to the preparations tested. It is important to note that green banana biomass does not interfere with the taste of food and brings numerous health benefits through the addition of fiber to food. Therefore, the green banana biomass added to the rice preparation with chicken cream obtained satisfactory acceptability, so it can be included in the school lunch menu.

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Biological activity from *Annona* genus fruit species seeds (Annonaceae)

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Abstract

Annonaceae Family has been characterized to exhibit a wide variety of biological activities (Aminimoghadamfarouj, Nematollahi, & Wiart, 2011). The *Annona* genus is the most abundance and the major importance because of its edible fruits. Studies on the antioxidant activity of this family are reported at the level of pulp and leaves, but there are few reports of seed activity (Barreca et al., 2011). Therefore, the aim of this study was to evaluate the antioxidant and cytotoxic activity of three *Annona* species cultivated in Colombia.

Plant material for this study was: *Annona muricata* L, *Annona cherimola* Mill y *Annona reticulata* L. (Figure 1) cultivates in region Cafetera of Colombia. The seeds were detached from the fruit and a pretreatment was performed according to previous studies (Castro, Alzate, & Guerrero, 2010), material was then degreased by means of the Soxhlet technique. Obtaining the extract were to passive maceration using ethanol as solvent. The Extract was then filtered and concentrated in a rotary flash evaporator and a stream of nitrogen (Giraldo & Guerrero, 2018). The antioxidant capacity was assessed using *in vitro* models for 1,1-diphenyl-picrylhydrazyl (DDPH) and ferric reduction activity potential (FRAP), moreover the total phenolic content (TPC) in the seed was determined using the Folin–Ciocalteu method (Scheme 1). A toxicity activity were conducted on brine shrimp, *Artemia salina* (Scheme 2) (Logarto, Silva, Guerra, & Iglesias, 2001).

Annona reticulata L extract was found to have the highest antioxidant activity for both methods, according to the FRAP (552.96 ± 3.61 mg TE/100 g of extract) and DPPH (333.61 ± 23.28 mg TE/100 g of extract). And TPC values of $236,47 \pm 27,69$ mg GAE/100 g extract. TPC was found to have a strong linear correlation with the antioxidant activity assessed using both methods, with FRAP ($r=0,88$) and DPPH ($r=0,88$). In toxicity evaluation of plant extracts by brine shrimp bioassay, an LC_{50} value lower than 10 $\mu\text{g/ml}$ is considered highly bioactive (Salazar & Soto, 2012). This is an indication of the fact that both the extracts were highly active (Giraldo & Guerrero, 2018). This study indicated that the seeds of *A. muricata* L, *A. reticulata* L and *A. cherimolia* L could be a natural source of antioxidant compounds. However, based on results of cytotoxic activity we conclude that extracts are quite promising regarding the evaluation the other pharmacological activities.

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Optimization of extraction method and chemical characterization of a hydroalcoholic extract of *Epilobiumangustifolium* L.

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Introduction

Epilobium species and their extracts are very popular in folk medicine for a wide range of applications. *Epilobiumangustifolium* L. is a Traditional Chinese Medicine plant used to treat traumatic injury, subduing inflammation and menstrual disorders. In Europe, extracts obtained from the aerial parts of *E. angustifolium* are commonly used to treat prostate diseases. Recent *in vitro* and *in vivo* research confirmed the beneficial activity of *E. angustifolium* in early stage of benign prostatic hyperplasia, inflammation of urethra and prostate, as well as micturition problems (Deng et al., 2018). These activities are mainly ascribed to flavonoids and ellagitannins (e.g. oenothain B), which have been identified in *E. angustifolium* (Baert et al., 2015). The present study aimed to: 1) optimize the extraction method from *E. angustifolium* aerial parts, in order to obtain a hydroalcoholic extract rich in polyphenols, through the Design of Experiment (DoE) approach; 2) determine the metabolic profile of the optimized extract.

Method

A systematic method consisting in the DoE was apply, in order to select the best experimental conditions for the hydroalcoholic extraction. The metabolic profile of the optimized extract was determined by using the latest generation chromatography, involving the use of Ultra-High-Performance Liquid Chromatography (UHPLC) coupled with a Quadrupole orbitrap Linear Trap (LTQ orbitrap), which allows for the detection of the compounds of interest at very low concentrations.

Results/Discussion/Conclusion

The present study lead to the determination of the best extraction conditions with the identification of the effect of solvent, pH, temperature and duration on extraction process.

The analysis of the chemical profile of the *E. angustifolium* optimized extract led to the identification of 43 compounds, including sugars, organic acids, non-flavonoids and flavonoids, both aglycone and glycosylated compounds.

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Antibacterial determination of *Justice spicigerain* cepas: *Escherichia coli* plyss 11229, *Lactococcus lactis* and *Staphylococcus aureus* ATCC 6538.

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Introduction

In recent years the study of medicinal plants has been resumed with greater force, in search of new active ingredients in response to the need to achieve effective alternatives for the control of bacterial infections. One of these plants is *Justice spicigera* commonly known in the region like muicle, muiltle or muite. Its common uses in the population are to purify the blood and for blood pressure, for problems of the digestive system, normally the stems and leaves of the plant from which an aqueous extract was made were used and it was tested in different bacterial strains such as *Escherichia coli* ATCC 11229, *Lactococcus lactis* and *Staphylococcus aureus* ATCC 6538.

Method

The disk diffusion methodology or method of Kirby Bauer was used, the antibiogram consists of depositing on the surface of the Mac Conkey agar of a Petri dish, previously inoculated with the microorganisms, sensidisks were impregnated in the different antibiotics Nitrofurantoin for *Escherichia coli* 11229, *Lactococcus lactis*, and Ceftriaxone for *Staphylococcus aureus* ATCC 6538., the same was done in the aqueous extract solutions of the *Justicia spicigera* plant at three different concentrations each trial, it was performed in triplicate, after 16-18 hours of incubation at 37 ° temperature, the sensidisks are surrounded by a zone of inhibition, of which they are read by measuring the radius of the halos where the case of each test is.

Results/Discussion/Conclusion

The results obtained in the antibiograms, forming inhibition halos for the three concentrations of the extract of the aqueous extract of the *Justicia spicigera* plant of 1, .250 and .500 µg / µL. The *Lactococcus lactis* strain shows sensitivity to the concentration of .250 µg / with an inhibition halo of 0.25 mm compared to the inhibition halos in the *Escherichia coli* 11229 strain that presented in the concentration 500 µg / µL with a radius of 1.17 mm , *Staphylococcus aureus* ATCC 6538 showed a concentration of 0.58 mm in the concentration 500 µg / µL, very noticeable inhibition halos were obtained in the positive controls where the specific antibiotic for each strain was applied; On the other hand, in the negative sterile water control, there were no notable inhibition halos compared to the positive control.

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Molecular docking of complexes formed between the compound UMSNH2014 with cyclodextrins

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Introduction

The increase in the enzymatic activity of the Aldose reductase (AKR1B1) is one of the causes of cataract formation in patients with chronic hyperglycemia (Browlee, 2001; Huang *et al.*, 2016). In the search of natural origin inhibitors of the AKR1B1 it was observed that the compound UMSNH2014 has inhibitory activity in tested *in vitro* and *in situ*. It was determined that this compound present cytotoxicity of concentrations higher than 40 μ M. The objective is predicted the formation of a molecular complex between the compound UMSNH2014 with cyclodextrins for the decremented the toxicity in the crystalline cells. The cyclodextrins are macrocyclics compounds formed by oligosaccharides united by α -D-(1,4) bond. In the pharmaceuticals industry are used to remedy disadvantages in the drugs such as lower solubility, low input on the cell and cytotoxicity (Del Valle, 2004).

Method

Molecular docking was performed for simulate to interaction between the cyclodextrin with the compound UMSNH2014. The structures of the cyclodextrins was obtained from ChemSpider. The structure of UMSNH2014 was designed and adjusted to its minimum conformation of energy in the program Maestro. A text file was written where is included the dates of the coordinates for the search of the interaction in the program. The molecular docking was performed in the program AutoDock Vina. Molecular docking between the cyclodextrin β and diclofenac sodium was realized as positive control.

Results/Discussion/Conclusions

Molecular docking between the cyclodextrin and diclofenac sodium a binding energy of -4.2 Kcal/mol was obtained and was one formed one hydrogen bond. The results obtained with the cyclodextrins and the compound UMSNH2014 is shown in the table 1. Molecular docking between UMSNH2014 and the cyclodextrin γ has positive binding energy (0.5 kcal/mol). This indicates it required additional energy for the reaction to be possible. That is to say this is negative for the formation of complex. The results obtained with the cyclodextrins α , β , γ δ and the compound UMSNH2014 showed formation of complex with the compound UMSNH2014. This is due to the hydrophobic environment of the cyclodextrin. This cyclodextrins were able to confine UMSNH2014. The complex formed between UMSNH2014 and the cyclodextrin δ was more stable.

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Table 1.-Molecular docking between the cyclodextrin and UMSNH2014		
Cyclodextrin	Hydrogen bonds	Affinity (Kcal/mol)
Alfa (α)	1	-1.8
Beta (β)	1	-3.8
Gama (γ)	2	0.5
Delta (δ)	1	-3.9

Production of phenols from the biodegradation of the avocado seed (*Persea americana* var. Hass)

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Introduction

The benefits that *Persea americana* brings to humans are not limited to the fruit (aguacate, avocado, palta). In traditional medicine it is used for the treatment of infectious diseases of the intestine, skin, and other illnesses. In cosmetics it is an important component of products for skin and hair care (Araújo et al., 2018). In addition to the consumption of the fresh fruit, it is processed to obtain guacamole. In this regard, Hass avocado production in Mexico in 2017 was 1 890 000 t. Guacamole production for 2018 was 78 863 t; which generated ~ 12 600 t of seed (16 % total fruit weight) (SIAP, 2019). Avocado seed is an important solid waste in the industrialization of avocado that requires attention. Hemicellulose, cellulose, and starch constitute mostly this waste. Lignin and other polyphenols limit the use of these polysaccharides in the generation of value-added products. Therefore, its biological degradation by microorganisms capable of producing ligninolytic enzymes to monomers is an alternative to chemical hydrolysis. The purpose of this study was to evaluate the production of phenol alcohols from seed by batch fermentation.

Method

Two intestinal bacterial isolates were obtained from dry wood termite *Incisitermes marginipennis*. A fungal isolate was obtained from stem-end-rot avocado fruit. Bacterial isolates were preserved and propagated in Muller-Hinton medium. The fungal isolate was propagated and preserved in malt agar and potato dextrose agar. Oxygen demand assay kits were used according to the manufacture's specifications. COD and BOD values were determined for each batch of avocado seed flour. Initially, bacterial ligninolytic capacity was determined by color degradation method in solid medium. The presence of *laccases*, lignin peroxidases, and manganese peroxidases were determined with ABTS substrates, veratryl alcohol, and manganese sulfate, respectively. Batch-type fermentations were carried out with microbial isolates and seed flour as a carbon source.

Results/Discussion/Conclusion

The two bacteria and the fungus were able to decolorate the dye. An indication that these microorganisms excrete some oxide-reducing activity. After fermentation the extracellular enzymatic activities of *laccases*, lignin peroxidases, and manganese peroxidases were detected. The microorganisms were able to use avocado seed flour as a carbon source. The detection of mono phenols is in process.



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Evaluation of enzymatic activity on immobilized systems of oxide reductase for xylitol production

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Introduction

Naturally, xylitol is found in small amounts in plant fruits, microorganisms, animal tissues, and the human body (Chi *et al.*, 2019). Xylitol is a five carbon polyalcohol, a sugar substitute that is used in food and pharmaceutical industries due to its properties such as anticariogenicity, preventive against otitis and respiratory infections. Xylitol is a molecule considered as a non-caloric sweetener, with sweetening power similar to sucrose and lower caloric value (2/3 of that from sucrose). The pattern of consumption of sweeteners is focused mainly on sugar and recently on non-caloric sweeteners. Global consumption of xylitol in 2013 was approximately 160 000 t, which is equivalent to a market value of 670 million dollars, and is expected to reach 242 t by 2020 equivalent to 1 billion dollars. Xylitol is industrially manufactured by catalytic reduction of xylose. This process is laborious, costly, and energetically demanding. Biotechnological production of xylitol offers an alternative that lowers the energetic requirement of the xylitol production process and its general cost. In this study we immobilized an oxide-reductase (OR) enzyme capable of converting the substrate xylose to xylitol. The proposed process is expected to aid in the decrease of the production cost for this polyalcohol because of the less energetically demanding reaction conditions.

Method

The oxide-reductase (OR) enzyme extract from rabbit lens was extracted via saline precipitation with ammonium sulphate 40 % (w/v). The method for the determination of enzymatic activity of OR is based on the measurement of consumption of NADPH, which is proportional to the enzyme activity. The enzymatic activity unit (IU) is defined as the change of 0.001 units of absorbance per minute; this change was measured using a spectrophotometer at 340 nm for two minutes. The enzyme glucose-6-phosphate dehydrogenase was added to the extract for the cofactor regeneration. The immobilization was performed with 45 mL of prehydrated dextran resin, packed in a chromatography column (22 x 250 mm), and equilibrated with 55 mM citrate buffer at pH 7 and 4 °C. The enzymatic complex was added with an equal volume of the same buffer.

Results/Discussion/Conclusion

The OR enzyme from rabbit demonstrated catalytic activity when using xylose as substrate, which infers that the enzyme is capable of producing xylitol. The reaction velocity of OR increased from 4.25 $\mu\text{mol}/\text{min}$ to 5.89 $\mu\text{mol}/\text{min}$ when the enzyme glucose-6-phosphate dehydrogenase was added. This indicates that the regeneration system for NADPH is functioning and OR has more cofactor available, and therefore, the

reaction velocity increases. The enzymatic complex was immobilized in dextran particles with a chromatographic column. No enzymatic activity was found in the samples eluded from the column, which indicates that the enzyme stayed in the support. In conclusion, the OR from rabbit lens was capable of consuming xylose as substrate, the cofactor regeneration system functioned, and the enzymatic complex was immobilized in dextran particles.

Acknowledgment: The authors are grateful for the partial funding of this research from the UMSNH and foundations MCCC and m3P.

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***Tagetes lucida* and the control of stem-end-rot in avocado fruit**

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Introduction

Stem-end-rot in avocado fruit is a postharvest disease caused by a fungal consortium. Restrictions to the postharvest use of agrochemicals in fruits motivates the search for new components of natural origin such as extracts and essential oils of medicinal plants with antimicrobial properties such as *Tagetes lucida* for the control of fungal infections (Espinoza-Madrigal et al., 2017). Edible coatings offer protection for fruits during postharvest. Due to its chemical configuration and characteristics such as abundance, low toxicity, hydrophobicity, biodegradability, biocompatibility and antimicrobial activity, plant extract is used to prepare biofilms, gels, microspheres and microcapsules (Souza et al., 2014). The aim of this work is show the sensitivity of fungi isolated of stem-end-rot from avocado fruit with an extract of the medicinal plant *T. lucida*.

Methodology

T. lucida plant was collected in its wild form and extracts of flower, fruit, leaf, stem and root were obtained with three solvents of different polarity (tl-extracts). The antifungal effect was evaluated with the fungi *Colletotrichum acutatum*, *Diaporthe phaseolorum*, *Phomopsis viticola* and *Oligoporus* sp. through the disk diffusion method (0.3 mg/μl) and the growth rate was determined (IG = ratio between pathogen growth in the treatment and pathogen growth in the negative control) on the fourth day after inoculation. The tl-extracts were fractionated by column chromatography. The major plant components was elucidate by nuclear magnetic resonance (NMR). The most effective extract *in vitro* was used and its toxicity was evaluated in *A. salina* (Meyer et al., 1982; Mejía Barajas et al., 2012).

Results / Discussion / Conclusion

Twelve tl-extracts were obtained and their antifungal potential was evaluated. Six tl-extracts were effective. The methylene chloride extract from flower showed the lowest IG with the fungi evaluated, so it was fractionated by column chromatography. Three coumarins were identified and purified. This tl-extract was used to assay the fungus sensitivity. *In vitro*, all of them inhibited the growth of all fungal isolates. The toxicity of *T. lucida* extract was evaluated with the *A. salina* model. The indicator to determine the safety of a substance in *A. salina* is 1 000 μg/ml. The LC₅₀ was 28.01 μg/ml, a lower value than acute toxicity tests established for this biological model. This value suggests that tl-extracts are safe for use in the control of stem-end-rot in avocado fruit.

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Identification of compounds with anti-inflammatory properties in *Lobelia laxiflora*

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Introduction

The poison ivy (*Toxicodendron radicans*) oily resin (urushiol oil) may cause a delayed type of hypersensitivity upon skin contact. The hypersensitivity response is characterized by redness, swelling, papules, vesicles, and blisters (Gladman, 2006). The symptoms slowly improve and disappear in most individuals after one to three weeks. Overall, the symptoms may range from mild to severe. In some extreme cases, a severe allergic reaction can lead to an anaphylactic shock. Usually the phyto dermatitis is treated with corticosteroids, which can frequently cause collateral health problems (Alsukait et al., 2017). Hence, the use of alternative therapeutic chemical compounds that do not endanger the patient's health is advised. Under this perspective, Mexican local traditional medicine uses a plant of the genus *Lobelia* to treat this inflammatory event (Bello et al., 2015). The purpose of this study was to extract and identify natural compounds with known anti-inflammatory properties from the local plant *Lobelia laxiflora*.

Method

The leaves obtained from *L. laxiflora* plants were dried and pulverized. Compounds were extracted with ethyl acetate three times by simple percolation. The extract was filtered using Whatman paper and concentrated on a rotary evaporator at 40 °C. Subsequently, the components of the ethyl acetate extract were separated by column chromatography, until all the major constituents were individually isolated. The isolated compounds were identified by nuclear magnetic resonance (NMR).

Results/Discussion/Conclusion

Five major compounds were identified in the *L. laxiflora* ethyl acetate leaf extracts: ursolic acid, oleanolic acid, β -amyrin, taraxerol, and lupeol. These correspond to previously reported triterpenoid secondary metabolites and are known to have anti-inflammatory properties. The stereoisomers ursolic and oleanolic acid were abundant. It is not unusual that several triterpenoid compounds with anti-inflammatory effects are found in plants that exhibit anti-inflammatory properties. For instance, *Ligustrum* sp. contains 5 to 6 anti-inflammatory-related triterpenoids; each species with different relative concentrations of each compound, possibly related to its anti-inflammatory effect (Wu, 2011). Interestingly, *Lobelia* and *Ligustrum* share 4 abundant anti-inflammatory triterpenoids. In conclusion, these results support the traditional use of *Lobelia laxiflora* as an anti-inflammatory therapeutical plant. The evaluation of the anti-inflammatory capability for each of the five identified triterpenoids in phyto dermatitis caused by urushiol is in process.

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Biodirected characterization of antioxidant compounds of *Jatropha dioica* originary from Durango

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Introduction

The use of medicinal plants assumes a high value in the world due to the properties attributed to it, among which antioxidant properties aimed at disease prevention and control due to the growing dissatisfaction of the population towards traditional medicine stand out, due to lack of success in treatments and side effects.¹ An efficient and low-cost alternative is the use of plants in the development of new agents for disease prevention, control and treatment.² Therefore, the aim of this study is to characterize the compounds with antioxidant capacity of extracts of *Jatropha dioica* originary of Durango.

Method

Experimental study in which extracts of the solvents were obtained: hexane, methanol, ethanol, ethyl acetate and water, separated in aerial and underground part, phytochemical screening was performed to identify different metabolites. The antioxidant capacity was determined by the ABTS³ method and the reference curve was performed with ascorbic acid for comparison.

Results/Discussion/Conclusion

The extracts presented alkaloids, terpenes, phenols, carbohydrates and flavonoids. In addition, the hexanic extract had a presence of resins, the underground methanolic part presented resins, reducing sugars and amino acids, the aerial part presented resins and reducing sugars, the ethanolics presented reducing sugars, in the underground part of ethyl acetate there was presence of polyuronides and resins, in aerial part resins, the aqueous presented saponins, reducing sugars and amino acids. Cortés et al. in 2005 collected the plant in Hidalgo and found a low concentration of alkaloids and triterpenes, average saponins and no flavonoids, however, in our extracts we find these metabolites, this may be due to the collection area, validating the concept of phenology, where factors such as temperature, water stress, soil conditions and climate influence the concentration and availability of metabolites, explaining the differences of this species.⁴ For antioxidant capacity, methanolic and ethanolic extracts from underground showed greater capacity with 94.36 and 94%, followed by aqueous part air with 92.96%. Martínez and cols in 2014 found the presence of flavonoids and terpenes, indicating them as possible responsible for the antioxidant effect for aqueous and methanolic extracts, the extracts where we found greater antioxidant capacity, in addition to ethanolic extract, the

presence of these metabolites being evident through of the screening carried out, they also found that the hexane extract was negative for antioxidant capacity while in our study we found a 40% capacity for this extract, this may be due to differences in collection and extraction of the plant that influences the presence and conservation of its compounds.⁵ Ramírez et al. in 2016 collected the plant in Coahuila and found that the antioxidant capacity was higher in root, which coincides with our results since two of the extracts presented greater capacity in underground part.⁶ According to the results, *Jatropha dioica* from Durango is considered a potential source of metabolites that provide antioxidant capacity, useful in the prevention and treatment of various diseases, it is also important to highlight that there are variations in the availability and concentration of metabolites depending on the phenology.

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Manufacture of transfemoral prosthesis with expandible polystyrene

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Introduction

According to the World Health Organization (WHO), almost 200 million people experience difficulties in its operation, in some years the disability will be a cause of even greater concern, because its prevalence is increasing. One of the alternatives with increasing use are prostheses. The purpose of this project is to manufacture a transfemoral prosthesis with foaming polystyrene in an recovered state as an alternative to people with ablation effects, giving priority to the physical and mental well-being of those who require it.

Method

The methodology was based on the manufacture of specimens with expandable polystyrene recovered together with the starch and strands of threads separately, whose purpose is to establish comparisons between the properties of each under mechanical tests, using the stress-strain curve in the linear state of the specimen, this for the implementation of a single material that will constitute each of the pieces for the fabrication of the prosthesis.

For the manufacturing process of the transfemoral prosthesis, materials such as recovered expandable polystyrene were used, which was collected through physical appearance.

Results/Discussion/Conclusion

Thanks to the properties presented by styrene at the time of production, a phase inversion can be generated, which initially consists of the production of high impact polystyrene, a reaction medium consisting of a single phase where polybutadiene reacts with styrene, as styrene reacts; The polystyrene begins to precipitate in a lower percentage of conversion, and given a different phase to the polystyrene dispersed in the production of high impact polystyrene, the particles grow more numerous, exceeding the particles of the initial phase. Transient and ideal moment where the arrangement of the phases is reversed; in which the initial phase becomes the continuous medium to form another type of polystyrene.

The analysis made it possible to know the possibility of using a solid residue such as expandable polystyrene, this material is economical, in addition to being reused it can reduce the impact on the environment. Creating a new culture towards solid urban waste.

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Characterization of the antihypertensive effect of a mixture of citroflavonoids

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Introduction

The *Citrus* genus is characterized as one of the largest accumulators of phenolic compounds such as glycosylated flavanones and polymethoxylated flavones [Khan et al., 2014]. Epidemiological evidence and clinical and pre-clinical studies suggest that flavanones have a positive influence in cardio-metabolic parameters, preventing cardiovascular diseases (CVDs) [Chanet et al., 2012, Cassidy et al., 2012]. Previous studies indicate that the sweet orange's major flavanones are hesperidin (H) and naringenin (N) which are particularly concentrated in the peel (albedo and flavedo) rather than the pulp [Khan et al., 2010; Cabañas, 2013]. González-Rivero 2018 reported synergic vasorelaxant effect of H:N mixture (mix-H:N). This effect was endothelium-dependent through increase of nitric oxide in *ex vivo* assay of vascular smooth muscle [González-Rivero, 2018]. The aim of the present study was to determinate acute and subchronic cardiovascular effect through hypotensive ability in hypertensive rats with the proposal to contribute to the growing knowledge about citroflavonoids for the treatment and prevention of CVDs.

Method

The flavonoids were purchased of Sigma Aldrich Co. Blood pressure changes were evaluated using the noninvasive tail cuff system Panlab Harvard Apparatus, LE 5007. Adults spontaneous hypertensive rats (SHR) and Wistar Kyoto rats (WKY; control group) were randomly divided into four groups of six animals each (n=6). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) values were obtained at the beginning of the experiment (T0). Then, for the acute assay the group I and II were orally administered with 161 mg/Kg of the mix-H:N or distilled water (5 mL/kg) respectively, later the SBP, DBP and HR values were obtained at 1, 3, 5, and 7 hours post-administration. In the subchronic assay the group III and IV were orally administered either distilled water or mix-H:N for four weeks respectively and measurements of SBP, DBP and HR were obtained every week. The results were represented by response-time curves and these were expressed as mean \pm standard error of the mean (SEM) of SBP, DBP or HR values. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values less than 0.05 were considered statistically significant respect to control group.

Results/Discussion/Conclusion

On average, acute orally administration of mix-H:N (161 mg/kg) in SHR caused a significant decreased respect to control (WKY group) in the SBP (8.78% and 7.57%) and DBP (12.3% and 11.35%) at the 5 and 7 hours respectively post-administration. Oral administration of mix-H:N (161 mg/kg per day) continuously

decreased the SBP and DBP of SHR during the experimental period. The mix-H:N caused a decreased in the SBP (15.79%) and DBP (11.26%) in the first week, and these effects were maintained during four weeks (SBP: 23.16% and DBP: 19.59%). The heart rate values were not modified in the acute or subchronic experiments. The mix-H:N significantly decreases the systolic and diastolic blood pressure in hypertensive rodents. These data provide an added value to the pharmacological description of the citroflavonoids mixture.

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An engineered yeast for the production of resveratrol: a preliminary approach

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Introduction

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) with a broad spectrum of beneficial properties, including antioxidant, anti-inflammatory, and chemopreventive effect. Resveratrol is considered as a promising compound as a dietary supplement, functional food ingredient, cosmetics additive and as a therapeutic agent, because of its nutraceutical properties. The amount of resveratrol in grape peel and peanuts is very low which limits its extraction. This fact represents a challenge for the scaled obtainment of this compound. Metabolic engineering using genes from novel plants or optimized genes, represents a plausible alternative to achieve the production of this valuable compound.

Method

The first attempt to reconstruct resveratrol biosynthesis in yeast (*Saccharomyces cerevisiae* INVSc1) was done by inserting phenylalanine ammonia lyase (*AtPAL2*), cinnamic acid hydroxylase (*AtC4H*) and p-coumaroyl-CoA ligase (*At4CL2*) from *Arabidopsis*. To complete the final biosynthetic step, resveratrol synthase (GenBank: EF620775) from peanut (*Arachis hypogaea*) was also inserted. The engineered yeast was designed to overexpress the *AtATR2* from *Arabidopsis* in order to improve the electron interchange as previously reported (Li et al., 2016). The engineered strain (IN1223) was subjected to fermentation process ($OD_{600}=0.8$) at 30 °C for 10 hours in glass flasks (2.5 L) under moderate shaking (200 rpm) in order to obtain the final product using 20 g L⁻¹ of glucose as a carbon source.

Results/Discussion/Conclusion

The recombinant strain of *S. cerevisiae* was able to produce resveratrol 82.6 mg L⁻¹ after 10 hours. Interestingly, kinetic studies on the production of resveratrol by the strain IN1223 suggested that approximately 50% of the yields were obtained at 3 hours, 70% at 6 hours and 90% at 8 hours. Previous studies using similar genetic material but using resveratrol synthase from *Vitis vinifera* were able to produce over 100 mg L⁻¹ of resveratrol but, employing complementary strategies of metabolic engineering. Further tests using diverse copies of resveratrol synthase from peanut, codon optimization and insertion of non-coding regulatory RNA sequences should be explored. The obtained strain represents the initial prototype for the further enhancement of resveratrol production.

Acknowledgment. NVR thanks CONACyT-Mexico for the project 578.

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Isolation, viability and count of splenocytes in a murine experimental model

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Introduction

Spleen aspirates contain differentiated cells from the six strains, which can be made microscopically viable according to a standardized protocol.

Objective: Standardize the viability and count of splenocytes using spleen samples from mice in a murine experimental model.

Materials and methods: We worked with spleens of 23 female mice distributed in 6 groups: G1 (3 healthy mice), G2 (8 mice infected with *Strongyloides venezuelensis* (Sv) larvae), G3 (control mice of the group that received the AA0029 Immunomodulator and the adjuvant *Quillaja Saponaria*), G4, G5 and G6 control mice of the groups that received recombinant proteins 14-3-3, major and Ferritin respectively. They were sacrificed on day 8 post infection. The spleen was removed, in a sterile petri dish with 10 ml. of PBS., 5 ml was inoculated. at each end. Tissue residues containing pale red splenocytes were obtained, 5 ml was collected in order to make the count.

Results: The formula was used: amount of splenocytes counted in 4 fields / height x dil x area, being the height 1/10, dilution 1/20 and the area: 4. Replacing we have the factor 50. For the calculation we had: Amount of splenocytes x Total Vol x Final Vol x factor (50).

Conclusions: The splenocyte count allows us to determine the levels of IFN γ , IL-2, IL-4 and IL-10 cytokines from primary cultures induced with the peptide in vitro, evaluation of immune response by lymphoproliferation, as well as in investigations in immunology, the characterization of the antigens can be defined.

Key words: Splenocytes - murine experimental model - viability

EM is a fellow of the Carolina Foundation, Government of Spain.

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Anti-inflammatory effects of *Eryngium maritimum* aqueous extract on Jurkat cells

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Introduction

Eryngium maritimum L. is a perennial herbaceous plant, belonging to the family of Apiaceae which is widely distributed in the Mediterranean countries. The genus is known to contain many bioactive compounds including acetylenes, flavonoids, coumarins and triterpene saponins. Infusion of aerial and root parts of this species are used in folk remedies as antitussive, diuretic, anti-edema and as coadjuvant in wounds and burns for its anti-inflammatory properties.

Method

The anti-inflammatory effects of hydrophilic extract obtained from the roots of *E.maritimum* were evaluated *in vitro* using Jurkat cells. Cells stimulated with phytohemagglutinin (PHA) were treated with the extract in two different non-toxic concentrations (100 and 500 µl extract/ml medium) evaluated with the MTT assay.

Results/Discussion/Conclusion

The concentrations of interleukin 2 (IL-2), tumor necrosis factor alpha (TNF- α) and nitrite determined in the culture medium after 4 and 24 hours of treatment evidenced a significant increase after stimulation. The release of both cytokines and nitrite was ameliorated by the extract treatment in a dose-dependent response. The gene expression of nuclear factor $\kappa\beta$ (NF- $\kappa\beta$), TNF- α , cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) measured after 4 hours of treatment followed the same pattern of response. The expression of these genes was increased after PHA stimulation and partially reduced in cells exposed to the

plant extract. In conclusion, the hydrophilic extract of *E.maritimum* roots exerted anti-inflammatory effects on Jurkat cells by reducing cytokine and nitric oxide release and the expression of pro-inflammatory genes.

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Antifungal activity of *Andira inermis* (fabaceae) heartwood extract and medicarpin against some xylophagous and phytopathogenic fungi

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Introduction

The plant secondary metabolites are an alternative of synthetic fungicides used to control phytopathogenic fungi (1). This study aims at investigating the effect of the extractable of *Andira inermis* ('W. Wright' Kunth ex DC.) heartwood and medicarpin (2), a phytoalexin derived of this, against three wood-destroying fungi (*Trametes versicolor*, *Phlebiopsis* sp and *Irpex lacteus*) and two plant pathogens (*Alternaria solani* and *Fusarium solani*).

Methods

Fragments of heartwood of *A. inermis* were dried and ground to yield powder and extracted by soxhlet method, at 70°C for 6 h under reflux with 1000 mL ethyl acetate per 60 g of sample. After extraction the content was concentrated using rotary evaporator at 40 degrees under vacuum and the extractable was prepared to 10 mg/mL in methanol. (+)-Medicarpin was purified by column chromatography and their identification was made by ¹HNMR mass spectroscopy (2). Data were recorded on a Varian Mercury-400BB spectrometer (400 MHz for ¹H and 100 MHz for ¹³C NMR) in CDCl₃ with TMS as internal standard. Antifungal activity was carried out by the diffusion assay in Wells on agar (3) with different concentrations with both extractable and purified medicarpin (0.05, 0.1, 0.25, 0.5, 0.75 y 1 mg/L) using methanol as dissolvent, a negative control (methanol) and Benomyl® (5 mg/mL) as positive control, incubated at 8 days, at 25° C in dark conditions. The results obtained in the different tests were expressed through the mean ± standard deviation, which processed through an analysis of variance of one way and differentiation of averages (Tukey, p < 0.05, n = 3), through the JMP8 software.

Results/Discussion/Conclusion

After 8 days of incubation, a greater antifungal effect was observed in the xylophagous fungi that in the phytopathogenic fungi, with both extractable and (+)-medicarpin. At this time, a higher antifungal effect of extractable and medicarpin was observed in *T. versicolor* and *Phlebiopsis* sp with 100% inhibition at concentrations from 0.25 mg/mL and inhibition below 50% at the concentration of 0.125 mg/mL. However, the extractable and (+)-medicarpin inhibited 100% mycelia growth of *I. lacteus* with 0.75 and 1 mg/mL, respectively, and showed lower antifungal activity on *F. solani* and *A. solani* with 50 to 60% inhibition,

respectively, at the maximum tested concentration (1 mg/mL). Medicarpin has proven to have antifungal activity, purified of *Butea monosperma* crust inhibited the growth of *Cladosporium cladosporioides* (4), from *Dalbergia monetaria* wood showed inhibition on *Rhizoctonia solani* and *Helminthosporium teres* (5). It is also reported with antifungal activity against *T. versicolor* (2).

Both the extractable and (+)-medicarpin, obtained from *A. inermis* heartwood showed the greatest antifungal activity on xylophagous fungi (*T. versicolor* and *Phlebiopsis* sp), with the lowest values of CMI, CL50 and CL100 (data no shown). The inhibition on *F. solani* and *A. solani* was lower than observed on xylophagous fungi.

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Relationship between the antioxidant, anti-inflammatory activity and polyphenolic content of avocado oil, *Potentilla indica* and *Hedeoma piperita*

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Introduction

Avocado oil, *Potentilla indica* (Rosaceae) and *Hedeoma piperita* (Lamiaceae) have secondary metabolites as phenolic compounds with a potential of antioxidant and anti-inflammatory property. However, little is known about these activities in *in vitro* studies and its relationship with the content of bioactive compounds present in them. This study analyzed the antioxidant activity and anti-inflammatory of methanol fraction of native Mexican avocado (*Persea americana* var. *drymifolia*) seed oil, chloroform fraction of *P. indica* fruit methanol extract and of *H. piperita* infusion fresh leaves, to compare with the total content of phenolic acids and flavonoids.

Methods

Avocado seed oil was obtained by Soxhlet method (6 h reflux with hexane/100g fresh weight) at 70 °C, fractionated by liquid-liquid extraction with absolute methanol (AvFMeOH); the chloroform fraction (PiFCHCL3) was obtained through a liquid-liquid extraction of a mature fruit extract of *P. indica* by maceration in methanol (4 °C); the infusion of fresh leaves of *H. piperita* (HpInf) was obtained with 20 g of leaves/L of boiling water (at 95-100 °C for 10 min). The content of total phenolic acids and flavonoids was obtained by spectrophotometric methods (1,2). In addition, the antioxidant activity *in vitro* (%) was determined by the DPPH(3) and ABTS(4) methods using 1 mg/mL each fraction. Finally, the anti-inflammatory activity (%) was analyzed using carrageenan-induced paw edema in rat model (5), with topical administration of 50 µL each fraction at different doses (0.1, 1 y 10 mg/kg).

Results/Discussion/Conclusion

The avocado methanol fraction (AvFMeOH) achieved a 99.7% of antioxidant activity with ABTS method (IC₅₀ 0.035 mg/mL), finding the highest values for total phenolic acids and flavonoids (0.17 µmol EAG/mg w.f., 5.49 µmol EQ/mg w.f., respectively). The chloroform fraction (PiFCHCL3) presented the highest antioxidant activity with 67.25% (DPPH) and 44.51% (ABTS), presenting the highest values of total phenolic acids (0.615 micromoles Eq. Acid Gallic/g fresh weight). *H. piperita* leave infusions (HpInf) presented highest percentages of fresh leaves with values of 90% (DPPH) and 93% (ABTS), with high content of phenolic acids (0.25 µmoles EAG/mg F.w.) and flavonoids (7.69 µmoles EQ/mg F.w.). With anti-

inflammatory assays, showed that AvFMeOH (1 mg/kg) had the greater anti-inflammatory effect at 8 h of treatment with 72.21%; at this time, the PiFCHCL3 (1 mg/kg) presented an anti-inflammatory effect with 77.8%, and HpInf showed 50% of anti-inflammatory activity with a dose of 1 mg/kg. This activity was similar to that observed with the topical administration of ibuprofen (12.5 mg/kg), which presented a 78.8% of anti-inflammatory activity. A direct relationship of the content of bioactive compounds with antioxidant and anti-inflammatory activity was observed in each fraction.

Zavala-Guerrero B., Sereno-Villaseñor L.A. and Raya-Ramírez J.O. acknowledges the scholarship from CONACyT.

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The Mediterranean Dietary Pattern and its Role in Human Health and Longevity

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Introduction

Food is a common, essential and vital necessity for human survival. However, food can be considered more than just the aspects mentioned above, as it is interpreted in different ways varying from region to region. The Mediterranean diet particularly plays in the individual an important role related health aspect. The study aimed a systematic review based on scientific evidence correlating the adoption of the Mediterranean dietary pattern and aspects related to human health and longevity.

Method

The research is a literature review conducted with scientific studies available in databases including Google Scholar, PubMed, SCOPUS. The relevant keywords associated with Mediterranean diet were searched in combination with keywords associated with longevity, health, food culture and population studies. The reference list of articles was limited to Portuguese and English languages and publications ranged from 2001 to 2016.

Results/Discussion/Conclusion

The UNESCO's recognition of the Mediterranean diet as Intangible Cultural Heritage of Humanity in 2007 was due its importance for health promotion through healthy habits. Several factors are related between health, longevity and culture of the Mediterranean diet, including the potential ability in prevent chronic diseases such as cardiovascular disease and increased immunity also are pointed as benefits acquired by Mediterranean diet consumers. The Mediterranean diet is complex and rich with a variety of colors and flavors, including the variety of foods high in cereal, olive oil, low in red meat and the moderate consumption of wine during meals were the key factors responsible for Mediterranean diet benefits. The populations of the Mediterranean region have enormous knowledge of foods, produce their own food rather than import, so food is available and affordable. The Mediterranean diet meets and balances all human food needs, as indicated in the food pyramid model for nutrition security. This balance denotes several benefits, such as helping to regulate body weight against obesity, being a priority in cancer prevention cancer especially those of digestive tract and, consequently, promoting longevity, environmental and social sustainability. However, the most important aspects of this dietary pattern are that it serves to promote a healthy form of eating as well as encompassing the history, culture and art of well-being of the people who share it.

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Cuniculture: Benefits and Challenges of Meat from Alternative Specie

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Introduction

Cuniculture has been present in human life since the Modern Age. Documents show that European and Asian civilizations already used rabbits in the eighteenth and nineteenth centuries. In the national context, the implementation of this raw material occurred only in the twentieth century, however production was restricted only in some products. Only from 1980 the production of rabbit meat was stimulated. The study aimed to review the benefits of rabbit meat consumption as a functional food.

Method

The present study opted for a literature review conducted with scientific studies available in databases including Google Scholar, PubMed, SCOPUS. The relevant keywords associated with cuniculture were searched in combination with keywords associated with alternative meat, alternative species, health benefits, rabbit meat and functional food. The reference list of articles was limited to publications from 2012 to 2016.

Results/Discussion/Conclusion

Rabbit meat besides being a tasty meat has excellent nutritional profile with a good profile of polyunsaturated fatty acids, low levels of fat and cholesterol as well as relatively high concentrations of proteins and iron compared with beef and chicken. Cuniculture has a sustainable character and is also a strategic activity, because the production of this food generates a meat of high nutritional quality in a short time, and a high productivity with a low economic impact. Although it's a meat with a high protein content and its possibilities of consumption with easy digestibility of nutrients besides pleasing the taste of national cuisine, it is a meat that still has a cultural barrier. This challenge is related to some variants, including the disorganization of the sector. Importantly is a low availability of rabbit meat to consumers as well as knowledge about their nutritional values. Although it's a meat of high quality, flavor and culinary acceptance, the studies under analysis highlight the challenges of rabbit breeding in Brazil are still crucial to its development. Given the historical context, the arrival of this practice is recent in Brazil, therefore the studies showed the possibilities to expand and favor the development of an advanced culture of sustainability and nutritional dissemination of consumption of meat from alternative species as rabbit meat in a healthy diet.

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Anti-herpetic activity of Colombian Labiatae family essential oils on strain human Alphaherpesvirus type 1 aciclovir resistant

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Introduction

The Human Alphaherpesvirus type 1 (HHV-1) and Human Alphaherpesvirus type 2 (HHV-2) are enveloped viruses with a double-stranded DNA genome that belong to the family *Herpesviridae*. HHV is usually associated with skin and epithelial mucosa infections, causing various diseases, such as gingivostomatitis, herpes labialis, meningitis, and ocular and genital infection. The treatment of choice is mainly based on nucleoside analogs among these the acyclovir (ACV), which act as competitive inhibitors of naturally occurring nucleotides and their prolonged use, has led to the emergence of resistant viral strains. Essential oils are complex aromatic-smelling mixtures of various compounds such as monoterpenes and sesquiterpenes hydrocarbons, alcohols, aldehydes, ethers, and ketones as well as different phenylpropene derivatives. For medical and commercial use, essential oils are derived from plant material by hydro- or steam distillation. The goal of the present study is the evaluation of the antiviral activity of twenty seven essential oils from plants of the families Verbenaceae, Piperaceae, Labiatae, Zingiberaceae, Rutaceae, Poaceae, against an isolated HHV-1 ACV-resistant for incubation of the virus with essential oil before cell adsorption.

Method

The antiviral activity of essential oils on Vero E6 cells against one TCID₅₀ of HHV-1 strain 29R resistant to ACV were carried out using the end-point titration technique (EPTT). Two-fold dilutions of the essential oils (12.5–100 µg/mL) and viral suspension of one TCID₅₀/ 0.1mL were mixed and incubated for 15 minutes at room temperature before they were added to confluent monolayer Vero E6 cells then it was incubated at 37 °C in a humidified 5% CO₂ atmosphere for 48 h. Finally, medium was removed and cell monolayers were fixed with a solution of formaldehyde (3.5%) and crystal violet (0.4%).

Results/Discussion

In this study we found that the essential oils of *Minthostachys mollis* (Kunth) Griseb and *Salvia aratocensis* subsp. *Suratensis* with more antiviral activity showed a reduction factor (*Rf*: ratio of the virus titer in the absence over virus titer in the presence of the tested compound) of the viral titer of 1 x 10¹ in non-cytotoxic concentrations of 25 and 100 µg/mL respectively. The antiherpetic activity in other strains of HHV-1 (CDC Atlanta) and HHV- 2 (VR-734 G) sensitive to acyclovir and wrapped RNA viruses as CHIKV, of these essential oils have been reported. It is possible that the antiviral effect is the result by disruption of viral envelope, inactivating the viral particle before cell absorption and entry.

Conclusion

We suggest that these essential oils have potential as broad-spectrum antivirals, against viruses enveloped with DNA and RNA genomes, due to the alteration of the viral membrane or envelope by interaction with components of lipophilic composition of the oils. These results support the potential use of essential oils from medicinal plants as agents for the topical treatment of herpes labialis and herpes genitalis in patients with resistance to nucleoside analogs to prevent spreading of the infection, pain and inflammation control, and to shorten the duration of typical symptoms such as itching and burning.

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The use of *Camellia sinensis* and *Phaseolus vulgaris* for weight loss

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Introduction

Phytotherapy is characterized by treatment from medicinal plants and their different pharmaceutical forms which have been used for a long time. These natural products for medicinal purposes allow humans to reconnect to the environment, normalizing physiological functions, restoring immunity, promoting detoxification and rejuvenation. Evidence suggests that older civilizations used medicinal and toxic plants for more remote healing, prevention and treatment (Firmo *et al*, 2011). Obesity has now become a major public health challenge, along with its comorbidities whose health professionals are looking for new ways of effective treatment. Thus, herbal medicines can help in the treatment of various pathologies, including obesity. The herbal market has been growing, motivating pharmaceutical companies to seek scientific studies that prove their efficacy, safety and quality. The aim of this study was to evaluate the scientific evidence of the use of *Camellia sinensis* and *Phaseolus vulgaris* and their effects on weight loss.

Method

The work consisted of a literature review of articles published in the last twenty years (1996 to 2016). The keywords used were “herbal medicine”, “history of herbal medicine”, “legislation”, “weight loss” and “obesity”. In addition, a specific search was also performed for the following herbal remedies “*Camellia sinensis*” and “*Phaseolus vulgaris*”, the inclusion criteria were: specific search for study with “*Camellia sinensis*” and “*Phaseolus vulgaris*” in weight loss, which resulted in 27 articles. The search was performed in the databases Scielo, Lilacs, Medline, Pubmed and Google Scholar.

Results/Discussion/Conclusion

Clinical studies of 12 weeks to 10 years with the use of *Camellia sinensis* herbal medicines have shown a positive effect on weight loss. The major thermogenic effects are reduced body mass index, waist circumference, fat body mass and decreased skin folds. It is suggested that the action on weight loss is due to the presence of antioxidant compounds such as catechins present in the main components of tea, especially epigallocatechins. Phase 2 clinical studies in overweight or obese patients also are demonstrated effects on weight loss throughout treatment for a maximum of 60 days. Similar to green tea studies, participants also had lower triglyceride levels with the use of white bean extract (*Phaseolus vulgaris* supplement). The group consumed the extract up to 2 times before main meals, which revealed its effectiveness in weight loss compared with the placebo group. These effects are due the presence of the alpha-amylase inhibitor (phaseolamine) in white bean extract. It is important to highlight that there were adverse reactions such as



headache and abdominal pain with the use of this herbal medicine. However, for both supplements studied due to their low cost and easy access, herbal medicines need scientific support for the prescription and guidance of health professionals to their patients. Its improper use can cause damage to health, due to the active principles that can alter organic functions, as well as interfere with the action of drugs used simultaneously.

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BIOello Design

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Introduction

The jewel has played a leading role in the history of the costume since the oldest civilizations. The desire to adorn the person with jewelry is a characteristic common to all populations since the prehistoric age, when necklaces and amulets were made in organic materials, ivory, shell and bone (the oldest jewel in the world, the *Bomblos Necklace* dated by archaeologists 75,000 years ago it was made with 41 sea pearls, from a mollusk, the *Nassarius krassianus*). The great turning point in the evolution of the jewel came with the progress made in the processing of metal in the 3rd millennium BC, when in Mesopotamia, Egypt and the Greek world the first gold jewelry appeared. This heritage of ornamental techniques and repertory was passed on to the European West by the Romans and Byzantines. The minerals that were used in the most remote times for their beauty, for their splendor and brilliance, not only for their aesthetic value but also symbolic, superstitious, medicinal, spiritual and religious were amber, diaspro, coral, pearl, turquoise, emerald and jade. Currently, synthetic stones reproduced in the laboratory have been made that have exact correspondence in nature, same chemical composition, same structure, same hardness. *BIOiello design* proposes through a process of interaction between humans and mechanical and computer systems, ornamental artifacts with beneficial characteristics, designed and made ad hoc with natural or synthetic stones.

Method

"Every jewel and unique jewel is in fact replicable at will, even using less valuable or completely common materials (...) and the fact that if they produce a single specimen or tens of thousands (...) does not necessarily change the design approach (...) I think it's a good thing that we'll be able to do it," (cfr. D'Auria A., De Fusco R., *Il progetto del Design* 1992).

BIOello design uses interaction design actions: identify needs and establish requirements; develop alternative design proposals that meet the identified requirements; build interactive versions that can be communicated and evaluated; assess its acceptability.

Conclusion

BIOello design draws multifunctional jewel accessories for well-being through the deepening in the gemological field, experimenting with the use of precious synthetic materials that have the same chemistry, crystalline structure and equal hardness to the natural correspondent. *BIOello design* creates artifacts in line with the principles of sustainable design from the point of well-being, environmental and ecological aimed at the well-being of people, personalized jewelry, with craftsmanship: cameo, inlay, decoration, watermark, overhang, through interaction design actions.

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Evaluation of antioxidant capacity of *Micrantha Cav Tagetes*.

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Introduction

In oxidative stress reactive oxygen species (ERO) exceed the body's antioxidant defenses; These EROs (Finkel and Holbrook, 2000) are involved in the etiology of different degenerative diseases (arthritis, cancer, diabetes, among others) (Shahidi, 2004). Antioxidants are compounds that can inhibit or delay the oxidation of other molecules by disabling the initiation and / or propagation of free radical chain reactions.

The genus *Tagetes* includes species that have demonstrated their antioxidant capacity, among other medicinal properties (Barquín P. and Zamora L. 1991). This genus belongs to the Asteraceae family; In this work, the antioxidant capacity of *Tagetes micrantha* Cav plant was studied. from the State of Durango, Dgo.

Materials and methods

Wild plant material was collected in October 2018, in a rural town known as Los Llanitos, Durango, Mexico. It was identified in the herbarium of CIIDIR-IPN, Durango unit with the number of voucher 52303. Subsequently, the plant extracts were obtained (previously separated from the aerial part and land part of the plant) with solvents methanol, ethanol, acetate ethyl and aqueous; The solvent was removed by concentrating on a rotary evaporator and using lyophilizer. Phytochemical screening was performed for the determination of secondary metabolites. The antioxidant capacity was determined by the ABTS method.

Results, discussion and conclusions

In our study, most of the extracts presented the following secondary metabolites: alkaloids, reducing sugars, phenols and tannins, carbohydrates and flavonoids. The extracts that presented the highest antioxidant capacity based on the Trolox equivalent antioxidant capacity test were the root methanolic extract, aerial part ethanol extract, acetate part aerial extract and root acetate extract (117.13, 101.23, 108.79 and 117.86

equivalents of trolox in $\mu\text{g} / \text{ml}$ respectively), taking as a cut-off point to be considered with good antioxidant capacity, above 100 equivalents of trolox in $\mu\text{g} / \text{ml}$.

In a study by Sánchez Humala in 2017, the antioxidant activity and phytochemical march of the *Tagetes filifolia* chapters were evaluated and the results obtained were: phenols and quinones; The greatest antioxidant effect was obtained from the ethanol extract of the *Tagetes filifolia* plant at a concentration of 100 $\mu\text{g} / \text{ml}$.

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Immunomodulator Effect of Berberine in Human and Mice cells

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Introduction

Alkaloids are secondary metabolites of plants derived from the degradation of amino acids. Berberine is an alkaloid extracted mainly from *Berberis vulgaris*, and had been studied due to its wide spectrum of biological activities. Being the immunomodulatory effect of important interest. The present study aims to evaluate the immunomodulation of leukocyte cells in the presence of Berberine.

Method

Bioassays were performed using human leukocytes obtained from peripheral blood and murine macrophages. The cells were exposed to different concentrations of berberine and their cytotoxicity was evaluated by the MTT colorimetric assay. Subsequently, the secretion of cytokines and their transcripts was evaluated by ELISA and PCR by using the supernatant of the bioassays and the total RNA extracted from the cells. The expression of MHC-II molecules in the cells exposed to Berberine was also evaluated by flow cytometry.

Results

Our results shows that a low concentration of Berberine (3 µg / mL) a higher number of viable cells were by the MTT assay, This concentration was chosen for future studies. From the supernatants of the bioassays, the presence of TNF-α in the extracellular medium was evaluated, while by PCR the presence of the cytokines IL-1, IFN-α, IFN-γ, IL-6, IL-12 and TNF-α was observed. By means of flow cytometry, a change in the expression of surface markers was observed as the major histocompatibility complex molecule.

Discussion

Estos datos sugieren que Berberina induce la activación de macrófagos murinos, así como en leucocitos humanos. Our data suggest that Berberina induces the activation and secretion of proinflammatory cytokines of murine macrophages, as well as in human leukocytes.

Conclusion

The pharmacological properties of berberine are proposed as a potential effective therapeutic agents for the future treatment of autoimmune, cardiovascular and chronic degenerative diseases, among others. Future studies will be necessary to deepen the immunological mechanisms of modulation by this alkaloid.

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Antioxidant and hypolipemian effect of *Arthrospira jenneri* (spirulina) in *Mus musculus* Var. *swiss*

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Introduction

Today, natural products have played an important role worldwide in the treatment and prevention of diseases. Spirulina, blue-green planktonic algae, is gaining increasing attention due to its nutritional and medicinal properties, since it has essential amino acids and vitamins, because of this it is used in the production of nutritional supplements. (Ponce, 2013) (Abu et al, 2013)

Method

In vitro assay: DPPH and total phenolic compounds were performed. Subsequently, in vivo tests were conducted where 35 *Mus musculus* var. *swiss* males distributed in five groups. The white group received carboxymethylcellulose (CMC) orally and physiological saline solution 10ml / Kg intraperitoneally, the control group received CMC orally and triton 400mg / Kg intraperitoneally, the standard group received Atorvastatin at a dose of 10mg / Kg orally and triton 400 mg / kg intraperitoneally and problem groups I and II received the natural supplement of *Athrospira jenneri* in doses of 0.05g / 100g and 0.3g / 100g respectively. The treatment was administered orally for 10 days, where on day 9 of treatment triton was administered to all groups except the white group that was administered saline. Finally, on the 10th day of treatment, exanguination was performed to determine cholesterol and triglyceride levels, as well as catalase activity. (Campos, 2013)

Results/Discussion/Conclusion

The antioxidant capacity of *Arthrospira jenneri* was determined which was $32.86 \pm 3,702$ (ET / g) and 9.81 ± 0.197 (EAG / g). Cholesterol levels (mg / dl) were 83.52 ± 18.52 (white), 126.24 ± 8.77 (control), 92.92 ± 5.20 (problem I), 97.80 ± 19.51 (problem II) and 73.42 ± 3.82 (pattern). The average triglyceride levels (mg / dl) were 93.60 ± 4.99 (white), 125.89 ± 14.35 (control), 98.78 ± 0.071 (problem I), 116.25 ± 29.14 (problem II) and 82.12 ± 2.63 (standard). The average specific activity of catalase (U / Hb) was 9.02 ± 1.568 (white), 3.94 ± 0.713 (control), 5.34 ± 1.053 (problem I), 8.12 ± 4.759 (problem II) and 5.619 ± 1.851 (Pattern).

It is concluded that *Arthrospira jenneri* was shown to have a lipid lowering effect, at a dose of 0.05g / 100g it presented a significant decrease ($p < 0.05$) in cholesterol levels and a significant decrease ($p < 0.05$) in triglyceride levels, and at a dose of 0.3g / 100g it generated a significant increase ($p < 0.05$) in activity of catalase.

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Hypoglycemic activity of the hydroalcoholic extract of the bark of *Vallea stipularis* L.f. "ccencocoy" in Holtzman strain rats.

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Introduction

Predictions made by WHO indicate that global growth in the prevalence of mostly type 2 diabetic patients will reach 439 million people by 2030. The use of plants with medicinal benefits represents an option, such as the *Vallea stipularis* with hypoglycemic activity and become an economic alternative for producers in the region.

Method

Basic experimental research with DCA. The sample was collected in the district of Churcampa-Huancavelica, and subjected to hydroalcoholic extraction. The hypoglycemic activity was determined using the method of hyperglycemia induced with aloxane in rats according to Kameswara Rao et al., (1999). Using 35 male Holtzman strain rats distributed in seven groups with weight between 180 - 210 g, the target received distilled water 2 ml/kg of weight, the control aloxane 180 mg/kg, the standard glibenclamide 5 mg/kg, and insulin 4 UI/kg. The hydroalcoholic extract of *Vallea stipularis* L.f. was administered to the experimental group at doses of 100, 200 and 400 mg/kg.

Results

The phytochemical march according to Lock de Ugaz, shows presence of flavonoids, tannins, polyphenols, reducing sugars, anthocyanins and catechins. The hydroalcoholic extract of the bark of *Vallea stipularis* L.f. "ccencocoy" exerts a hypoglycemic effect, the dose of 400 mg/kg is statistically significant to glibenclamide. Thin layer chromatography shows that flavonoids are present in the ethyl aceto fraction and the chloroform fraction.

Discussion

The phytochemical march of the bark of *Vallea stipularis* L.f. shows the presence of flavonoids and phenolic compounds similar to that pointed out by Chaparro et al. (2016). Rivera in 2018, through Tukey's test found that glibenclamide and phenolic compounds are statistically similar, and that phenolic compounds isolated



from the rhizomes of *Zingiber officinale* Roscoe have hypoglycemic effect. The comparative effect of glibenclamide and insulin do not differ much between them. At a dose of 100 mg/kg of the extract there is no significant activity. On the other hand, the doses of 200 and 400 mg/kg do show therapeutic activity. Kwon et al. concluded that tyrosine kinase blockade is another mechanism by which quercetin has effects against diabetes.

Conclusions

The hydroalcoholic extract at 80% of the bark of *Vallea stipularis* L.f. "ccenccoy" has hypoglycemic effect, with greater percentage of effectiveness the dose of 400 mg/kg statistically significant.

The secondary metabolites present in the hydroalcoholic extract of the bark of *Vallea stipularis* L.f. are mainly flavonoids, phenols and cardiogenic glucosides.

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BIOCIDE EFFECT OF SAPONINS OF *Chenopodium quinoa* Willd ON LARVAS OF *Phthorimaea operculella*. AYACUCHO

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Introduction

Natural products of vegetable origin with insecticide activity are valid alternatives for pest control in substitution of conventional synthetic pesticides, since they do not generate resistance, undesirable effects on organisms and negative impacts on the environment.

Method

The study was basic with experimental design. The extraction of yellow quinoa saponins, quantification by spectrophotometry, phytochemical screening, evaluation of the biocidal effect and the mean lethal concentration were performed. Saponin solutions of 1, 2.5 and 5% (3 repetitions and their control in a DCA) were used. Larval mortality was evaluated at 8, 19, 28 and 38 days of exposure. For pathogenicity, 20 potato moth postures were used.

Results

At 155.4 µg saponin a 27% mortality of larvae was observed; at 388.5 µg 36%; at 1368.5 µg 30%; the mean lethal concentration (LC50) on larvae of *Phthorimaea operculella* was 13583.4 µg saponin, which allowed to know its biocidal effect, when increasing the concentration the percentage of mortality was also increased, but this difference was not significant ($p > 0.05$, $\alpha = 0.05$). Phytochemical screening of quinoa seeds showed that saponins and triterpenes are the most abundant.

Discussion

The extraction of saponins was carried out in 50% ethanolic solution and controlled temperature in the microwave (78°C); data coinciding with Zarate (2016), who used 50% v/v ethanolic solution at 78.4°C when



extracting saponins from different varieties of quinoa. Bonifaz evaluated the insecticidal activity of hydrolyzed and non-hydrolyzed quinoa saponine on *Drosophila melanogaster*, finding positive results of the biocidal action of hydrolyzed quinoa saponins on *Drosophila*, and also reports several plants capable of synthesizing secondary metabolites that have biological properties of great importance against pest insects (Bonifaz, 2010); results that when compared with those obtained in the work have some similarity, since no significant differences were found with the concentrations worked.

Conclusions

The main active metabolites of the plant are saponins and triterpenes, the toxic effect of quinoa seeds is attributed to saponins of the triterpenoid type. The LC50 of the saponins of *Chenopodium quinoa* Willd against larvae of *Phthorimaea operculella* was 13583.4 µg of saponin, which allowed to know its biocidal effect.

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Anti-CHIKV and anti-HHV activities of carboxylate organotin(IV) derivatives of kaurenic acids

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Introduction

CHIKV is a RNA genomearthropod-borne-Alphavirus from the Togaviridae family. CHIKV infection results in a dengue-like febrile disease, and like that DENV infections at present there are not licensed vaccines or effective antivirals approved for these diseases. HHV is a genomic DNA virus from the Herpesviridae family and causes frequent infections in immunosuppressed patients. The long-term administration of acyclovir (ACV) for therapy against HHV infections can result in the emergence of ACV-resistant strains. It is therefore urgent to develop new anti-herpetic compounds with mechanisms that differ from that of ACV. The kaurenic acids are tetracyclic diterpenes present mainly in the species of the Asteraceae and Annonaceae families. These plants are used in traditional medicine, and biological activities reported in vitro trials have demonstrated that ent-kaurenic acid and grandiflorenic acid are active as antimicrobials, anti-inflammatory, anti-parasitic. This study aims to find broad-spectrum antivirals that have both activity for DNA and RNA virus.

Method

Based on the combination principles of medicinal chemistry, six carboxylate Sn(IV) complexes were synthesized using the ent-kaurenic and grandiflorenic acids as ligands, as a strategy to prepare Sn(IV) compound. The antiviral activity against CHIKV and acyclovir-resistant HHV-29R of these Sn(IV) complexes were evaluated by TCID₅₀ assay (Tissue Culture Infective Dose) using the end-point titration technique (EPTT) with Vero E6 cells. Antiviral activity- two-fold dilutions of compounds (10^{-0.625} μM) and viral suspension of ten TCID₅₀/0.1mL were mixed and incubated for 15 minutes at room temperature before they were added to confluent monolayer Vero E6 cells and further incubated at 37 °C for 48 h. After 48 h of incubation at 37°C in a humidified 5%-CO₂ atmosphere, medium was removed and cell monolayers were fixed with a solution of formaldehyde (3.5%) and crystal violet (0.4%).

Results/Discussion

Most derivatives of evaluated series heads, ent-kaurenic and grandiflorenic acid, showed anti- CHIKV and/or anti- HHV activity higher than their series head, respectively. Structural studies led to the identification of (ent-kaur-9(11), 16-dien-19-oate) triphenyltin(IV) and [bis-(ent-kaur-9(11), 16-dien-19-oate)] diethyltin(IV) as promising candidates for possible antiviral agents. The antiviral activity was quantified through a reduction factor Rf defined as ratio of the virus titer in the absence over virus titer in the presence of the tested compound. According to the estimate of Vlietinck et al. (1995), a purified natural molecule is considered to have a relevant or moderate antiviral activity when the reduction factor (Rf) of viral titer is $\geq 1 \times 10^3$ or 1×10^2 , respectively. In this study, we found for [bis-(ent-kaur-9(11), 16-dien-19-oate)] diethyltin(IV) the non-toxic concentration of 1.25 μM , showed highest viral reduction factor of 1×10^3 for acyclovir-resistant HHV-29R; while that for the CHIKV the compound that showed highest viral reduction factor was (ent-kaur-9(11), 16-dien-19-oate) triphenyltin(IV) to the non-toxic concentration of 0.625 μM .

Conclusion

We suggest that both molecules are promising antiviral agents for control and/or treatment of CHIKV and ACV-resistant HHV infections. This is the first report about anti-CHIKV and anti-HHV activities of organotin(IV) derivatives of kaurenic acids. Our subsequent investigations are directed to the search of activated tetracyclic diterpenes present in the species of the Asteraceae and Annonaceae families for potential antiviral activity through the formation of organometallic complexes.

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