NPA
Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

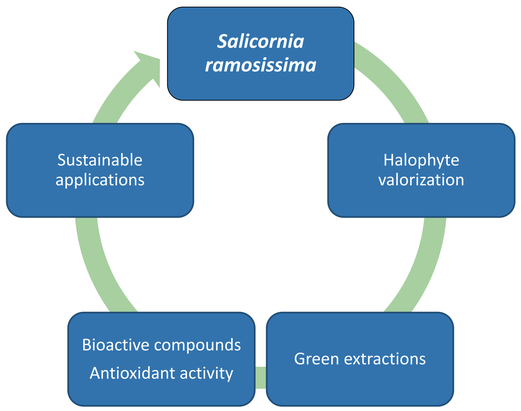
Microwave-assisted extraction of *Salicornia ramosissima* bioactive compounds: comparison with conventional extraction

Ana Margarida Silva[1], João Lago[1], Cristina Delerue-Matos[1], Francisca Rodrigues[1*]

[1]REQUIMTE/LAQV, Polytechnic of Porto - School of Engineering, Rua Dr. António Bernardino de Almeida, 431, Portugal.

[*]francisca.rodrigues@graq.isep.ipp.pt

Abstract



Introduction

Salicornia ramosissima

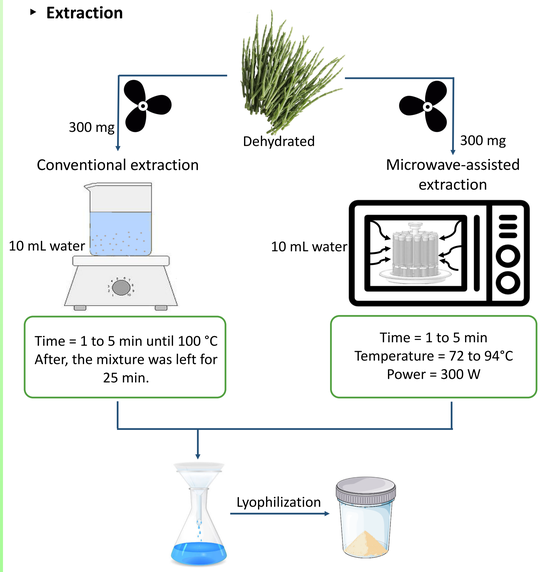
- Halophyte plant;
- Grows in saltmarshes, mainly in the coastline of Europe from the Arctic to the Mediterranean;
- Tolerates high salt concentration;
- In gastronomy, is an alternative to salt and the leaves are used for human;
- Presents antioxidant, anti-inflammatory, anti-diabetic and anticancer properties.

Microwave-assisted extraction (MAE)

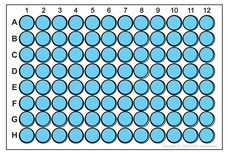
- Alternative to conventional extractions (CE);
- Green extraction method;
- Quick heating, lower solvent requirements, clean process and low cost;
- Water is a possible solvent used;
- Recover of high-added value compounds.

Materials and Methods

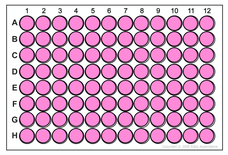
- **Extraction**



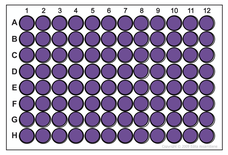
- **Characterization**



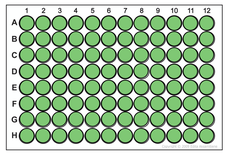
Total polyphenol content (TPC)



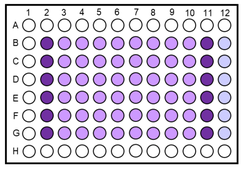
Total flavonoid content (TFC)



Ferric reducing antioxidant power (FRAP)



ABTS•+ radical scavenging activity (ABTS)



HT29-MTX viability through MTT assay

Results

Table 1 – Total phenolic and flavonoid contents (TPC and TFC, respectively), and *in vitro* antioxidant activities evaluated by FRAP and ABTS assays *of S. ramosissima* extracts prepared by CE and MAE. Values are expressed as mean ± standard deviation (n = 3).

Conventional extraction (CE); Microwave-assisted extraction (MAE); Gallic acid equivalents (GAE); Catechin equivalents (CEQ); Ascorbic acid equivalents (AAE); Ferrous sulphate equivalents (FSE).

S. ramosissima extracts	CE	MAE
TPC (mg GAE/g dw)	15.02 ± 2.01*	8.34 ± 1.22
TFC (mg CAE/g dw)	8.44 ± 0.45	8.41 ± 0.45
ABTS (µg AAE/g dw)	15.55 ± 0.78	17.74 ± 2.95*
FRAP (µmol FSE/g dw)	60.61 ± 6.64	65.56 ± 8.68

* In the same row indicate significant differences between extracts (p<0.05).

- For TPC and TFC, CE extract exhibited the highest values. The statistical analysis showed differences (p<0.01) in TPC assay between CE and MAE extracts.
- MAE extract demonstrated high antioxidant activity. For ABTS assay, the statistical analysis (p<0.05) revealed significant differences between extracts.
- For FRAP assay, the present study has similar results to *S. europaea* extracts obtained by ultrasound-assisted extraction (UAE) with 0 % of ethanol and better results than obtained by supercritical fluid extraction (SFE) [8]. However, *S. europeae* UAE and SFE extracts showed a best ABTS radical scavenging activity.

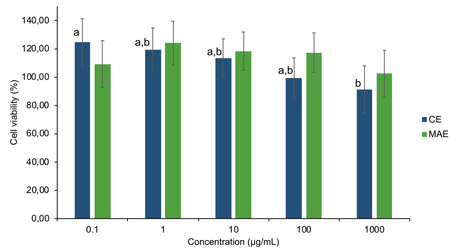


Figure 1 – Effects *of S. ramosissima* extracts prepared by CE and MAE on the cell viability of HT29-MTX at a range of concentrations between 0.1 and 1000 µg/mL. Different letters (a, b) mean significant differences between concentrations of the same sample (p<0.05).

- As shown in Fig. 1, MAE extracts did not lead to a decrease in the cellular viability of HT29-MTX. However, CE extract conducted to a viability of 91.20% after exposure to 1000 µg/mL.

Conclusion

- *S. ramosissima* is a sustainable source of antioxidant/antiradical compounds.
- MAE is an effective technique to recover antioxidant compounds from *S. ramosissima*.
- The values obtained may be due to the extraction time and temperature used. Besides this, abiotic/biotic factors influence the level of secondary metabolites in halophytes.
- Further studies should be performed to analyze the bioactive profiles, in order to select the best one for further cosmetic/nutraceutical applications.

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Acknowledgements

This work received financial support from project PTDC/ASP-AGR/29277/2017 – Castanea sativa shells as a new source of active ingredients for Functional Food and Cosmetic applications: a sustainable approach, supported by national funds by FCT/MCTES and co-supported by Fundo Europeu de Desenvolvimento Regional (FEDER) throughout COMPETE 2020 – Programa Operacional Competitividade e Internacionalização (POCI-01-0145-FEDER-029277). Ana Margarida Silva is thankful for the PhD grant from Portuguese Foundation for Science and Technology (SFRH/BD/144994/2019). The work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds as well as by the Applied Molecular Biosciences Unit-UCIBIO, which is financed, by national funds from FCT/MCTES (UID/Multi/04378/2019).

THE ANTI-INFLAMMATORY POTENTIAL OF HYDROLATES FROM TWO THYMUS SPECIES PRODUCED IN PORTUGAL

Ana S. Oliveira,[1,2] Joana Rolo,[1] Carlos Gaspar,[1,2,3] Rita Palmeira de Oliveira,[1,3,4] João P. Teixeira,[5,6] José Martinez de Oliveira,[1,2] Ana Palmeira de Oliveira,[1,2,3*]

[1]Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal; [2]Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal; [3]Labfit-Health Products Research and Development Lda, UBImedical, Estrada Nacional 506, 6200-284, Covilhã, Portugal; [4]Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3004-504 Coimbra, Portugal; [5]National Institute of Health, Environmental Health Department, Rua Alexandre Herculano 321, 4000-055, Porto, Portugal; [6]EPIUnit - Instituto de Saúde Pública da Universidade do Porto, Rua das Taipas 135, 4050-600, Porto, Portugal. *apo@fcsaude.ubi.pt

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

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Abstract

Background: Plants from the genus Thymus L. have been suggested to promote potential health benefits by their anti-inflammatory, and anti-proliferative, anti-microbial and anti-oxidant potentials [1,2]. Most studies focus on essential oils, but hydrolates, produced as co-products in the distillation process to obtain essential oils, can present several interesting applications. Therefore, we intended to explore the anti-inflammatory potential of *Thymus mastichina* and *Thymus citriodorus hydrolates*, both produced in Portugal.
Methods: *T. mastichina* and *T. citriodorus* hydrolates were obtained from Planalto Dourado and Ervitas Catitas, respectively. Their anti-inflammatory activity was investigated on LPS-stimulated mouse macrophages (RAW 264.7), by evaluating their effect on cellular viability (MTT assay) and on the production of nitric oxide (NO) using Griess colorimetric reagent. Additionally, hydrolates were also studied for their ability to scavenge NO, using (S)-Nitroso-N-acetylpenicillamine (SNAP) as a NO donor, in a non-cellular model.
Results: Both hydrolates affected cellular viability in a dose dependent manner, presenting cytotoxic effects only at the highest concentrations tested. Still, *T. mastichina* presented a higher biocompatibility, with a higher IC_{50} value (half maximal inhibitory concentration). Similarly to cellular viability, both hydrolates were able to inhibit nitrites production in a dose-dependent manner. Interestingly, significant reductions were observed at non-cytotoxic concentrations. Using a non-cellular model, *T. citriodorus* failed to scavenge NO and *T. mastichina* presented only modest scavenging activity, thus not justifying the high reduction of nitrites by LPS-stimulated macrophages.
Conclusion: Our preliminary results unveil an interesting anti-inflammatory potential of co-products of essential oil production, from two *Thymus* species cultivated in Portugal. This potential may be of interest to value hydrolates as active ingredients for different industries, particularly for the cosmetic industry, promoting an anti-inflammatory effect.

Plants Under Study

Hydrolates from *Thymus mastichina* and *Thymus citriodorus* were obtained from Planalto Dourado™ and Ervitas Catitas™, respectively.

Figure 1. A. *Thymus mastichina* plant from Planalto Dourado™.(Freixedas, Guarda, Portugal) B. *Thymus citriodorus* plant from Ervitas Catitas™ (Borba, Évora, Portugal)

Methodology

Cellular Viability and Nitric Oxide (NO) Production

Macrophages Culture (RAW 264.7)

LPS (1 µg/mL) + Hydrolates, 24h

Hydrolates, 24h

Medium Collection

MTT, 4h

Griess Reagent

Spectrophotometric evaluation
Cellular viability calculation

Spectrophotometric evaluation
Determination of NO stable metabolites

IC_{50} calculation
Sigmoidal dose-response (variable slope)

Scavenging Activity

Hydrolates ± SNAP (NO donor - 300µM), 3h

Griess Reagent

Spectrophotometric evaluation
Nitrites level quantification

Results

Cellular Viability

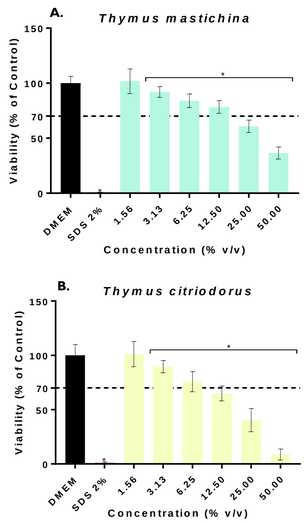


Figure 2. A. Effect of *Thymus mastichina* (A) and *Thymus citriodorus* (B) on cellular viability. Macrophages were plated and exposed to increasing concentrations of hydrolates for 24h. Negative (DMEM) and positive (SDS 2%) controls for cytotoxicity were also included. If the effect on cellular viability was above 70%, the correspondent concentration was considered non cytotoxic. The presented data correspond to the means ± SD of three independent assays and are represented as percentage (%) of control (DMEM). Statistical analysis: *t*-student test was performed for each concentration compared to control (DMEM); *p < 0.05 was considered a significant reduction.

Half Maximal Inhibitory Concentration (IC_{50})

Table 1. Estimated half-inhibitory concentration values (IC_{50} values) for *Thymus mastichina* and *Thymus citriodorus* Hydrolates, after cellular viability determinations. IC_{50} values were estimated using with GraphPad Prism (Sigmoidal dose-response, best fit values with 95% confidence interval).

Hydrolate	IC_{50}	95% CI	R^2
Thymus mastichina	33.38	30.29 to 37.11	0.8921
Thymus citriodorus	16.9	15.6 to 18.29	0.9091

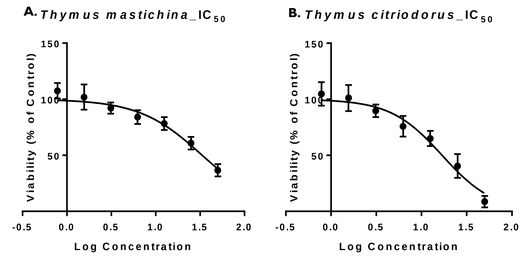


Figure 3. Graphical representation of A. *Thymus mastichina* and B. *Thymus citriodorus* sigmoidal dose-response curves, used to estimate half-inhibitory concentration values (IC_{50} values). The represented values correspond to the means ± SD of three independent assays. Concentrations (% v/v) are represented as Log of the concentration values.

Nitric Oxide Production

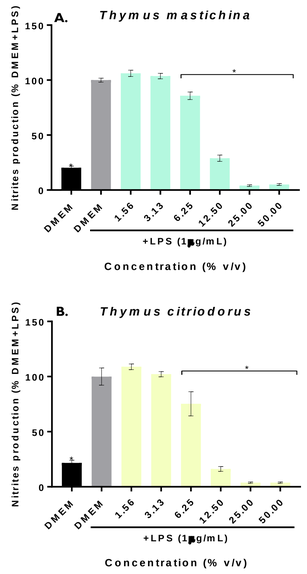


Figure 4. Effect of *Thymus matichina* (A) and *Thymus citriodorus* (B) on macrophage NO production upon an inflammatory stimulus. Cells were plated and exposed to DMEM medium or increasing concentrations of hydrolates for 24h, in the presence of LPS (1 µg/mL). Griess assay was performed to assess levels of NO stable metabolites (nitrites) in the cell supernatants. Data correspond to the means ± SD and are represented as % of control cells exposed to LPS (DMEM + LPS). A control without LPS was also included to evaluate basal NO production (DMEM). Statistical analysis: *t*-student test was performed for each concentration compared to positive control of NO production (DMEM+LPS). *p < 0.05 was considered a significant reuction.

Scavenging Activity

Table 2. NO scavenging capacity of *Thymus mastichina* and *Thymus citriodorus*. Results are expressed as a percentage of NO release triggered by SNAP. Data correspond to the means ± SD of three independent experiments and are represented as % of DMEM medium with SNAP (Control). A Control without SNAP was included to address basal nitrite values in the DMEM culture medium.

	Hydrolate Concentration (% v/v)	*Thymus mastichina* (Mean ± SD)	*Thymus citriodorus* (Mean ± SD)
+ SNAP (300 µM)	50	69.62 ± 5.263*	ND‡
	25	85.79 ± 1.623*	90.67 ± 6.021*
	12.5	93.24 ± 2.671	96.09 ± 9.682
	6.25	94.68 ± 3.378	99.91 ± 5.872
	3.13	99.69 ± 3.28	99.44 ± 8.738
	1.56	97.44 ± 2.457	97.33 ± 6.043
	Control	100 ± 7.63	100 ± 7.63
	Control wo SNAP	2.546 ± 1.079	2.546 ± 1.079

Wo: without. Statistical analysis: *t*-student test was performed for each concentration compared to control + SNAP ; *p < 0.05 was considered a significant reduction.
‡ Scavenging activity could not be determined due to an interference of SNAP with *Thymus citriodorus* hydrolate, at the highest concentration tested (50%).

Conclusions

Thymus mastichina and *Thymus citriodorus* hydrolates presented an interesting anti-inflammatory potential, demonstrated by a decrease in Nitric Oxide production, a pro-inflammatory mediator. Since this decrease seams not to be related with an intrinsic scavenging activity, paths of the inflammatory cascade may be involved. Further studies on specific inflammatory mediators should be pursued.
Due to their biocompatibility at effective anti-inflammatory concentrations, combined with their appealing fragrances, these products may be of interest as active ingredients for the cosmetic industries. Also, the presence of interesting bioactivities of hydrolates, such the ones here presented, can aid in the variolisation these co-products, typically produced in the distillation process to obtain essential oils.

Acknowledgements

This work was supported by "INOVEP project – Innovation with Plant Extracts", I&DT projects for companies in collaboration with scientific entities, project number 33815, Centro2020 and by Foundation for Science and Technology (FCT), through funds from the State Budget, and by the European Regional Development Fund (ERDF), under the Portugal 2020 Program, through the Regional Operational Program of the Center (Centro2020), through the Project with the reference UIDB/00709/2020. Financial support was also provided by FCT through PhD fellowship SFRH//BD/136192/2018. The authors thank Planalto Dourado™ and Ervitas Catitas™ for providing photographs of their *Thymus mastichina* and *Thymus citriodorus* plant productions, respectively.

AGROFORESTRY AS PRODUCTION SYSTEMS OF ESSENTIAL OILS

Ângela Pawlowski,[1*] Camila de Abreu Kuzey,[1] Kailany Panerai de Bastos[1]

[1]Instituto Federal de Educação, Ciência e Tecnologia Farroupilha *Campus* Santo Ângelo - Rio Grande do Sul, Brazil. *angela.pawlowski@iffarroupilha.edu.br

Abstract

Due to its several biological activities, essential oils are widely used in aromatherapy, healthcare and industry. Agroforestry systems are a land use system which integrate trees and agricultural crops as an alternative to make production sustainable. This study aimed to identify native Brazilian tree species which can be used in agroforestry systems proposing environmental preservation and sustainable management in order to produce essential oils. A bibliographic database analysis was conducted based on a list of sixty-three species. Three potential species to be managed on agroforestry systems in family farms to obtain essential oils are presented.

Introduction

Essential oils are complex mixtures of volatile substances derived from secondary metabolism of plants. Due to its several biological activities, these natural products are widely used in aromatherapy and healthcare, also being applied in the industry as flavourings, in pharmaceuticals, food, and cosmetics formulations [1].

Agroforestry systems are a land use system which integrate trees and agricultural crops [2]. The cultivation of medicinal plants, both shrubs [3] and woody plants [4] producing essential oils in agroforestry systems is already an alternative to make production sustainable.

Thus, this study aimed to identify native Brazilian tree species which can be used in agroforestry systems proposing environmental preservation and sustainable management in order to produce essential oils in family farms.

Methodology

A bibliographic database analysis was conducted based on a list of sixty-three species that have been used in the implantation of agroforestry systems projects related to environmental preservation and recovery of degraded areas developed by institutional partnerships in the South of Brazil.

Results

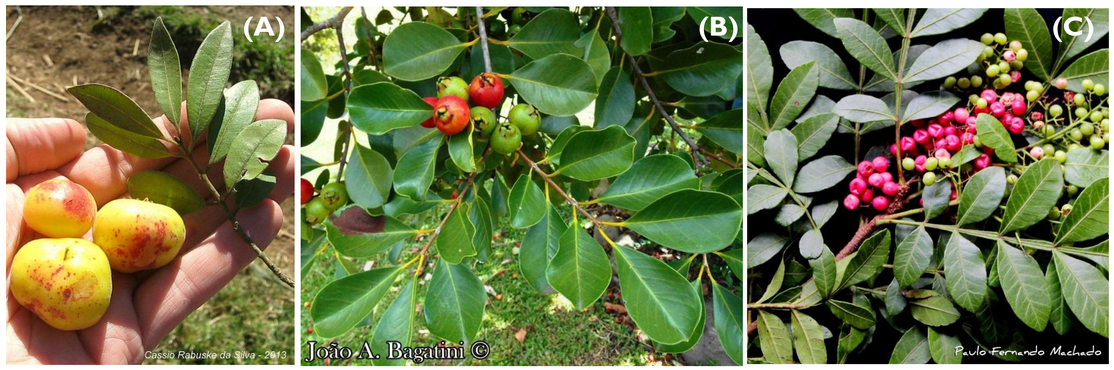
Three potential species to be managed on agroforestry systems to obtain essential oils are presented in Table 1. *Eugenia pyriformis* (Fig. 1A), *Psidium cattleyanum* (Fig. 1B) and *Schinus terebinthifolius* (Fig. 1C) fruits are already commercialized by family farmers; the first two species, in the production of juice pulps, and the third, as pepper (commonly known as Brazilian pepper).

The management of the agroforestry system, as leaf pruning, can be converted into the development of a new production chain. These essential oils may be used, for example, in the production of handmade soaps, an activity that is growing in the region. Thus, essential oils production constitute a new source of income for the rural producer.

Table 1: Potential species to be managed on agroforestry systems in family farms to obtain essential oils

Plant species	Plant part	Yield	Majoritary compounds
Eugenia pyriformis Cambess	fruits	1.23%	caryophyllene oxide (16.2%), limonene (12.4%) [5,6]
Psidium cattleyanum Sabine	leaves	0.26%	α-thujene (25.2%), 1,8-cineole (16.4%), β-caryophyllene (10.2%) [7]
Schinus terebinthifolius Raddi	seeds	2.66%	ρ-menth-1-en-9-ol (29%), hedicariol (11%) [8]

Figure 1: *Eugenia pyriformis* Cambess fruits (A), *Psidium cattleyanum* Sabine leaves (B) and *Schinus terebinthifolius* Raddi seeds [9].



Conclusion

Further research involves the seasonal chemical analysis of essential oil of these species growing in agroforestry systems and the development of technical guidelines on crop management to obtain essential oils.

This study proposes to find new sources of essential oils, understanding the productive potential of native trees, contributing to the development of local productive chains of sustainable forestry products.

Acknowledgements

Authors thanks the 'Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul' (FAPERGS) and 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq) for financial support to second and third authors, respectively. We also tkank Hitalo Ruan Ferrazza Antunes and André Camargo (Associação Regional de Educação, Desenvolvimento e Pesquisa - AREDE) for technical support.

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THE POTENTIAL OF THE MUSHROOM SHIITAKE (*LENTINULA EDODES*) AQUEOUS EXTRACT AS COSMETIC ACTIVE INGREDIENT

Bárbara Ribeiro[1], Aida Costa[1], Juliana Garcia[2], Ana Afonso[3], Conceição Fernandes[3], Fernando M. Nunes[4], Maria José Saavedra[2], Guilhermina Marques[2*]

[1]Floresta Viva- Chikioshira Lda., 4600-752 Amarante, Portugal, [2]CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, 5001- 801 Vila Real, Portugal, [3]CIMO - Centro de Investigação de Montanha, Polytechnic Institute of Bragança 5300-253 Bragança, Portugal, [4]CQ - Centro de Química, University of Trás-os-Montes e Alto Douro, 5001- 801 Vila Real, Portugal. *gmarques@utad.pt.

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

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ABSTRACT

Skin infections occur commonly and often present therapeutic challenges due to the growing concerns regarding multidrug-resistant bacteria. Herein, mushrooms could be a promise source of new and bioactive cosmetic ingredients with several properties. In fact, the mushroom shiitake (*Lentinula edodes*) has been used in different cosmetic formulations with several properties such as firmness, hydration and whiteness. Medicinal products from mushrooms could be used in cosmeceuticals, applied topically, such as creams and lotions, or nutricosmetics, ingested orally.

In this context, aqueous extract of the mushroom shiitake was examined concerning to antimicrobial activity against to clinical wound bacterial isolates as well as to antioxidant activity. The results showed an effective antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) [1], suggesting a potential use as antimicrobial ingredient in antiseptic cosmetic formulations.

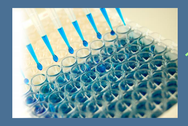
Based on these findings an organic cream was formulated with the purpose of acting as antiseptic on wounds and skin cracks, providing healing and nourishing the skin. The organic cream containing shiitake aqueous extract as antiseptic agent was enriched with selected organic vegetable oils containing nourishment properties and organic essential oils as healing and skin regenerating components.

INTRODUCTION

Numerous mushrooms and their ingredients have been known to be beneficial to the skin and hair. Mushrooms present many compounds (phenolics, polyphenolics, terpenoids, selenium, polysaccharides, vitamins, and volatile organic compounds) with excellent antioxidant, anti-aging, anti-wrinkle, skin whitening, and moisturizing effects, which make them ideal candidates for cosmetics products [2] . Nowadays, there is a growing interest for cosmetics containing natural and/or organic ingredients which make natural products as mushrooms an invaluable and exceptional source of them. Shiitake mushroom has several applications in cosmetics, ranging from an exfoliant to an anti-inflammatory, encourages faster skin renewal, and increases skin elasticity as a skin brightener [2]. To the best of our knowledge, there is no report on antiseptic products containing shiitake-derived extracts. Therefore, the present work aimed to explore antimicrobial and antioxidant activity of different Shiitake extracts.

METHODOLOGY

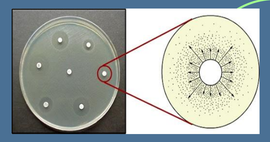
BIOLOGICAL AND CHEMICAL ASSAYS



Antioxidante activity ABTS assay

PHYTOCHEMICAL ANALYSIS

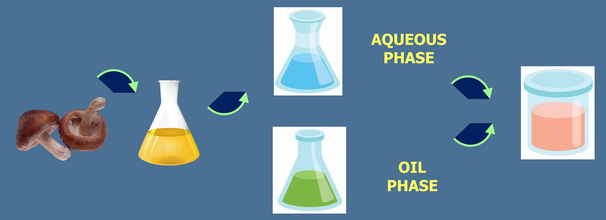
ANTIMICROBIAL ACTIVITY



Disk diffusion assay

CREAM ELABORATION

1. The water phase, contained the aqueous shitake extract is heated gently until 55°C.
2. Simultaneously, the ingredients of the oil phase (sweet almond oil, St. John´s Wort oil and beeswax) are heated gently in a separate container at the same temperature.
3. When both phases get to the required temperature the oil phase is poured gradually into the water phase.
4. The mixture is whisked vigorously using a mixer and a creamy texture is formed.
5. When the temperature of the cream drops down to around 40°C, the heat sensitive ingredients (lavender essential oil, calendula essential oil, vitamin E and preservative) are added and stirred in for a few more minutes to ensure uniform dispersion.
6. The cream, which was left overnight to cool to room temperature, was then filled into sterile containers and sealed.



RESULTS

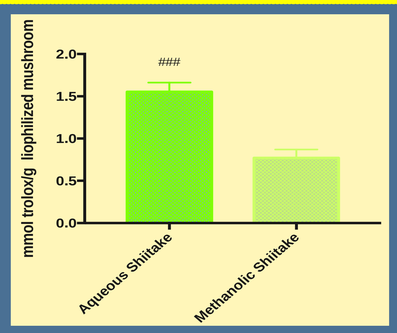


Fig. 1
ABTS scavenging activity of the aqueous and hidromethanolic extracts of Koshin Shiitake mushroom (### p<0.001)

Fig. 2
Cream containing antiseptic properties by Shiitake mushroom aqueous extract

*Table 1 – Antimicrobial Activity determined by the diameter of inhibition zones (mm)

Isolate	Methanolic Shiitake (mm)	Aqueous Shiitake (mm)
Methicillin-resistant Staphylococcus aureus MJMC511	6 ± 0,0	10 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC 025	6 ± 0,0	8 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC 027	6 ± 0,0	7 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC102	6 ± 0,0	12 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC111	6 ± 0,0	10 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC507	6 ± 0,0	10 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC534 B	6 ± 0,0	16 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC 539	6 ± 0,0	9 ± 0,0
Methicillin-resistant Staphylococcus aureus MJMC 552	6 ± 0,0	9 ± 0,0

CONCLUSIONS

The cream based in *L. edodes* aqueous extract seems to be of good value in disinfection and care of wounds and skin cracks due to its high antimicrobial activity and because the nourishing properties from the other constituents. The cream obtained in these work presents good texture and homogeneity and it has maintained its good appearance since its elaboration. Future work will be primarily focus on the prediction of the safety of the cosmetic product, namely in what concerns to skin sensitization, skin penetration, phototoxicity and cytotoxicity, according to the European cosmetics regulations, before its introduction on the market.

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ACKNOWLEDGEMENTS

This work was supported by the project I&T Companies in Co-Promotion FungiTech, Norte-01-0247-FEDER-033788; National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020 (CITAB-Center for the Research and Technology of Agro-Environmental and Biological Sciences), Centro de Química – Vila Real (UIDB/00616/2020) and UIDB/00690/2020 (CIMO-Centro de Investigação de Montanha).

FungiTech

DEVELOPMENT OF A COSMETIC ANTI-AGING GEL FORMULATION WITH EXTRACTS OF SPONTANEOUS PLANTS AND CULTIVARS OF HUMULUS LUPULUS L.

Briolanja dos Santos,[1] Olívia R. Pereira,[2] Andrea F. Afonso,[3] Luís Pedro,[4] Susana M. Cardoso[3] and Maria João Sousa[2,*]

1School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal; 2Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal; 3LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Portugal; 4Centro de Biotecnologia Vegetal (CBV), Lisboa, Portugal; *joaos@ipb.pt

Introduction

Hop (*Humulus lupulus* L.) is known worldwide as an essential flavor in the beer industry. Its major compounds have been demonstrated to be associated to health benefits, due to its antimicrobial, antioxidant, anti-inflammatory and anticancer activities [1].

It is also essential to use effective preservatives, and there are aromatic and medicinal plants with antimicrobial activity, as is the case with *Thymus zygis* which is widespread throughout the world, and its flowers and leaves are used, which have antimicrobial properties for bacteria gram positive and gram negative in addition to antioxidant capacity [2].

Figure I: The *Humulus lupulus* L .

Figure II: The *Thymus zygis*

Aim

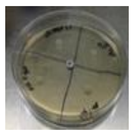
- Develop an anti-aging cosmetic gel by incorporation of different percentages of hydroalcoholic extracts obtained from cones and leaves of hop spontaneous and of the Cascade and Polaris cultivars and essential oil of T. *zygis*.
- Evaluate the stability of an anti-aging cosmetic gel.
- Phytochemical profile determination of hop extracts and of essential oil of thyme.
- Antimicrobial activities evaluation of hop extracts and of essential oil of thyme.

Materials and Methodology

A methylcellulose gel formulation was used as base to develop the hop cosmetic product by incorporation of hydroalcoholic extracts (1,25%; 2,5% and 5%) obtained from cones and leaves of spontaneous hop and of the Cascade and Polaris cultivars and essential oil of thyme;

The ability to inhibit the microbial growth was performed by:

- microdilution broth method;
- the diffusion in agar test;



The phenolic profile of hop extracts was determined by:

- UHPLC-DAD-ESI-MS^2 [3];

The composition of thyme essential oil was done by:

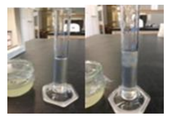
- GC and GC-MS;

The physical-chemical stability tests were:

- Organoleptic;
- pH;

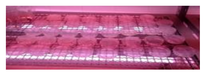
- Temperatures and humidity;
- Spectrophotometric;

- Extreme temperatures;
- Density;



- Mechanical vibration;
- Centrifugation;

- Texture;
- Color;
- Light;



Results

Thyme Essential Oil Composition

- ✓ The yield of the essential oil of *T. zygis*, based on the dry mass of the plant, was as follows 1.14%;

Table I: Main compounds of *T. zygis* essential oil

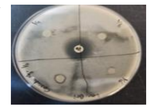
Compounds	Mean values (%)
terpinen-4-ol	25.8
Carvacrol	43.60
p-cymene	24.10
trans-sabinene hydrate	15.80

Antimicrobial Activity

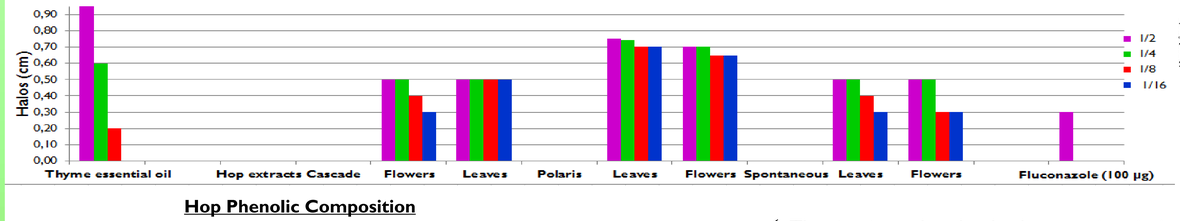
Table II: Antibacterial activity of *T. zygis* essential oil

	T. zygis (mg/ml)	
	MIC	MBC
Bacteria		
Escherichia coli	0,31	0,31
Staphylococcus aureus	0,031	0,031
Pseudomonas aeruginosa	1,25	1,25

- ✓ Hops and oil have antifungal activity against the yeast *Candida albicans*:

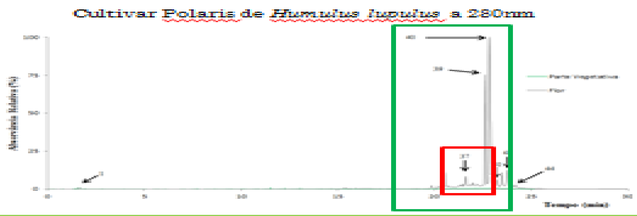


Graphic I : Antifungal activity of Hop extracts and T. *zygis* essential oil



Hop Phenolic Composition

- ✓ Hop cones were rich in phenolic compounds such as cohumulone, humulone (green) and xanthohumol, (red) which have been claimed as possessing anti-wrinkle effect [4];

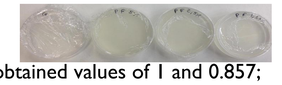


Stability of cosmetic formulations

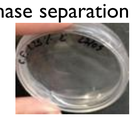
Any alteration/change in the analyzed samples:

- ✓ Temperatures and humidity;
- ✓ Extreme temperatures;
- ✓ Mechanical vibration;
- ✓ Centrifugation;

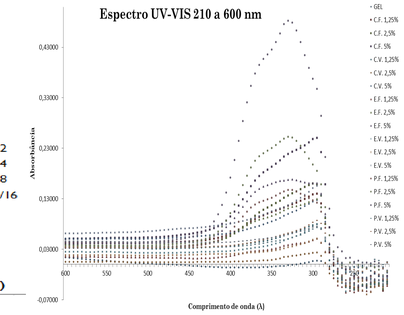
- ✓ There was not a large **color** variance with different concentration of extracts;



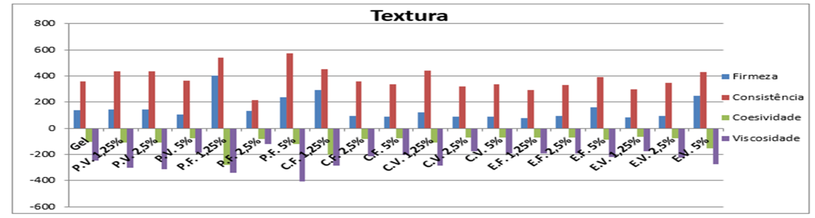
- ✓ The **relative density** obtained values of 1 and 0.857;
- ✓ In the **light test** there was phase separation in the samples due to the occurrence of dehydration;



- ✓ The **pH** of the formulations were slightly acidic;
- ✓ The **organoleptic characteristics** of the gel are refreshing, transparent in color, spread well and dry quickly and easy to remove;
- ✓ The **spectrophotometric test**, we found that the maximum peak is the Polaris gel in the cones at 5% with an absorbance of 0.482;

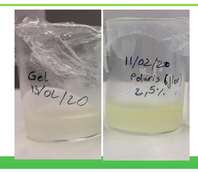


- ✓ The **texture** by the back extrusion it is possible to verify that the Polaris formulation has higher consistency values and lower viscosity;



Conclusion

Overall, the incorporation of hydroalcoholic extracts of hop and essential oil of thyme in methylcellulose gel formulations, especially with the 2.5% hydroalcoholic extract of Polaris variety cones, allow to obtained a stable cosmetic with anti-aging potential take into account his chemical composition.



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Acknowledgements

Thanks to FCT (Portugal) and the ERDF under the PT2020 Program for financial support to CIMO (UIDB/00690/2020) and LAQV-REQUIMTE (UIDB/50006/2020). SMC acknowledges the research contract under the project AgroForWealth (CENTRO-01-0145-FEDER-000001).

LIPID OXIDATION IN COSMETIC CREAMS: THE POTENTIAL PROTECTIVE EFFECTS OF NATURAL EXTRACTS

Inês Sousa, Cristina Soares*, Cristina Delerue-Matos, Maria João Ramalhosa, Valentina F. Domingues

REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal.

Abstract

Skincare products are functional products, consisting of multiple ingredients with several functions. Traditionally, one of the main ingredients are lipids that can be saturated or unsaturated. These unsaturated lipids and their interaction with the other components of the skincare products and external factors allow understanding and predicting the occurrence of oxidation. Lipid oxidation is a spontaneous and inevitable phenomenon and one of the most significant problems of deterioration of cosmetic products, affecting the chemical, physical, and sensorial features. Thus, it is essential to study the product's behavior during its lifetime, based on scientific and reproducible methods considering the presence of flower and fruit extracts.

In this work, skincare products of different compositions (with and without natural extracts) were tested, stored at 30ºC, in the absence of light, for six months. During each month, a set of samples (from A to J) was taken. Sensory analysis (color, odor, texture, and appearance changes) was made. For each skincare product, the primary oxidation products were determined by the conjugated dienes (DC) method expressed as a percentage and by the Peroxide index (Pi) method expressed in meqO_2/kg dry sample. Secondary oxidation products were also determined using the *p*-Anisidin Value (*p*-A) and the 2-Thiobarbituric Acid Reactive Substances (TBARS) test, expressed as malonaldehyde concentration (mg/g dry sample).

It was concluded that the presence of flower extracts influences the results obtained. The skincare products that have the greatest extent of lipid oxidation, based on the results obtained from Total Oxidation Value (TOTOX), is the Cream J with no natural extract in its composition followed by cream H that has fruit extracts. On the other hand, Cream D is the one with the smallest extent of lipid oxidation, having as a component *Calendula officinalis* flower extract. The presence of saturated fatty acids inhibits the process of lipid oxidation and decreases the oxidation rate. It can also be suggested that additives, such as flower extracts, can act as antioxidants preventing further degradation.

Introduction

Cosmetics are produced in order to be applied to healthy skin to protect it and improve its appearance, therefore contributing to the well-being of its users. Around 70% of the raw materials used in cosmetic creams are lipids (plant oils, fatty acids and alcohols, etc.) or derivatives of lipids (emollients, emulsifiers), and it is these that make the emulsion stable and rich and, in many cases, effective [1]. Lipids and their derivatives form a protective barrier in the skin and in the cosmetic emulsions, however, the oxidative potential of emulsions is often overlooked. Oxidation impacts the overall quality and safety of these cosmetic emulsions. Lipid oxidation is a spontaneous and inevitable phenomenon and one of the most significant problems of deterioration of cosmetic products, affecting the chemical, physical, and sensorial features. Products of these oxidation reactions then continue to pose serious harm to cells and can trigger other physiological oxidation reactions [2].

Thus, emulsions with lower lipid peroxidation are more stable and will reduce the negative effects of oxidation. The use of preventive measures during the formulation of emulsions are important. Many naturally occurring and cost-effective substances that possess low oxidation tendencies and confer oxidative protection can be used in emulsions. Sources of natural ingredients can include herbs, fruits, flowers, leaves, minerals, water and land [2]. The cosmetic formulations usually contain various combinations of many plant extracts and oils, for example, green tea, rosemary, grape seed, basil grape, blueberry, tomato, acerola seed, pine bark, and milk thistle. These vegetables extracts contain natural antioxidants, that is, polyphenols, flavonoids, flavanols, stilbens, and terpenes [3].

The objective of this work is to assess to potential antioxidant effect and lipidic oxidation protection, of several natural extracts present in commercial cosmetic creams (Table 1) using different methodologies to evaluate the formation of primary and secondary oxidation products [4].

Table 1. Presence of natural extracts in commercial cosmetic creams

Cosmetic cream	Natural extract	Function
A	Honey	moisturizing
	Camellia Sinensis leaf	antioxidant
B	*Aloe barbadensis*	moisturizing
	Viola tricolor	emollient
C	*Lycium barbarum* fruit	antioxidant
	Zingiber officinale root	antioxidant, emollient
D	*Calendula officinalis* flower	antioxidant, emollient
E	*Aloe barbadensis* leaf	moisturizing
	Carrageenan (*Chondrus crispus* extract)	emulsifier
F	*Rubus idaeus* fruit	emollient
	Ribes nigrum fruit	antioxidant, emollient
	Rubus fruticosus fruit	fragrance
G	*Pyrus malus* fruit	moisturizing
	Panicum miliaceum extract	emollient
H	*Fragaria ananassa* fruit	emollient
	R. idaeus fruit	emollient
	R. fruticosus fruit	fragrance
I	*Rosa x damascena* flower water	moisturizing
J	No natural extract	---

Methodology

Determination of the primary oxidation products: 1) Conjugated Dienes (CD) and 2) peroxide index (Pi).

$$DC\ (\%) = 100 \times \frac{Abs_{272nm}}{Abs_{232nm}}$$

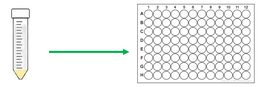
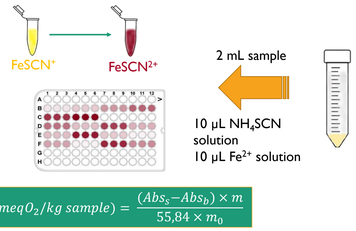


Figure 1. CD analysis

Peroxide Index (Pi)– *International Dairy Federation* (IDF) 74:2006



$$IP\ (meqO_2/kg\ sample) = \frac{(Abs_s - Abs_b) \times m}{55{,}84 \times m_0}$$

Figure 2. Pi analysis

Determination of the secondary oxidation products: 3) p-Anisidine Index (p-A) and 4) 2-Thiobarbituric Acid Reactive Substances (TBARS).

$$ipA = \frac{10(\times\ 1{,}2 \times Abs_d - Abs_a)}{m_s}$$

Figure 3. p-A index analysis

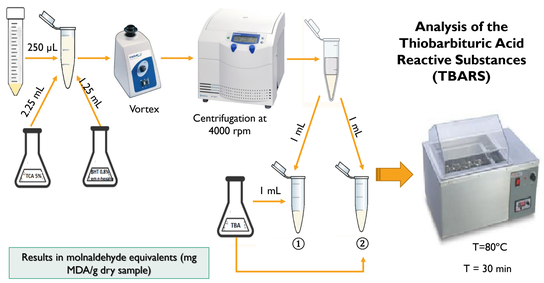


Figure 4. TBARS determination

Determination of Totox Value

$$Totox\ Value = 2(Pi) + p\text{-}A$$

Results

The determination of the primary and secondary oxidation products of the cosmetic creams are presented in Figure 5. These products were analyzed when opening the packages (month 0) and after 6 months.

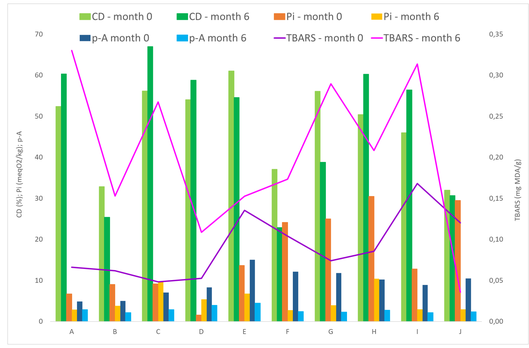


Figure 5. CD (%), Pi (meqO_2/kg), p-A and TBARS (mg MDA/g) in cream samples when opened (month 0) and after 6 months of use (month 6).

After 6 months:

- Samples A, C, D, H and I show an increase in the CD ratio that can be related with an increase in the oxidation process and the formation of secondary products (λ = 272 nm) and a decrease in peroxides (conjugated dienes λ = 232 nm). Although, very complex mixtures like creams can have strong absortion at 232 nm interfering with the results.
- Samples C and particularly D have an increase in the Pi indicating that the oxidation process is probably in the first stages. The decrease in the Pi in the other samples may indicate that hydroperoxides have already been degraded forming secondary oxidation products (aldehydes).
- Samples A, B show the lowest p-A value followed by samples C, D and I. Anisidine reacts with 2 double bonds (conjugated) aldehydes formed during the degradation of hidroperoxides.
- Sample D shows the lowest value for TBARS followed by samples E, B and F, 6 months after opening the cream packages. TBARS are related with the secondary oxidation products formed from the degradation of hidroperoxides.

The Totox value for each cream is presented in Figure 6.

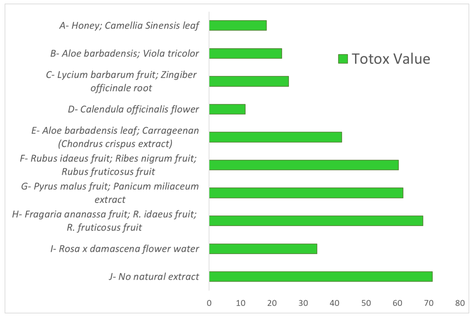


Figure 6. Totox value for each cream and relation with the cream composition in natural extracts

Sample D is the one that presents the lowest Totox value followed by sample A, B, C and I. Considering these samples natural extracts, all these samples have he addition of flower or plant extracts. All the other samples have a higher Totox value and in common have the addition of fruit extracts, or no natural extracts.

Conclusion

- **In general, the presence of natural extracts seems to have a positive impact on the lipidic protection against oxidation.**
- **Samples with natural extracts obtained from flowers (*Calendula officinalis*, *Viola tricolor* and *Rosa x damascena* seems to protect lipids against oxidative degradation when considering Totox value and TBARS.**
- **Samples with extracts obtained from fruits or with no natural extracts' addition seems to suffer a greater extent of lipid oxidation.**
- **Totox value can be considered as the correlation between the peroxide index that represents the degradation potential and aldehydes that can represent the effective deterioration state of the lipids. Considering this, the sample with the highest lipid stability is sample D (sample with *Calendula officinalis*).**
- **Sample J is the one with the highest Totox value indicating its highest degradation status.**

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Acknowledgements

The authors acknowledge the funding received through REQUIMTE/LAQV, National Funds (FCT, Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2019.

NPA
Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

TYROSINASE AS A PROTEIN TARGET FOR DERMATOLOGICAL TREATMENT

Diogo Pinto Luís,[1] Filomena Adega,[2] Rui M.V. Abreu[3]

[1]Escola de Ciências da Vida e Ambiente, Universidade de Trás-os -Montes e Alto Douro (UTAD), Quinta de Prados 5000-801, Vila Real, Portugal; [2]Departamento de Genética e Biotecnologia, Universidade de Trás-os -Montes e Alto Douro (UTAD), Vila Real, Portugal; [3]Centro de Investigação da Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia 5300-253, Bragança, Portugal. Email: diogo.pinto.luis@hotmail.com

Abstract

Tyrosinase can be considered the most important enzyme for the synthesis of melanin. Alterations in the formation of melanin has been related to several dermatological disorders ranging from simple hyperpigmentation to skin cancers. Tyrosinase inhibition may be of great interest to prevent melanogenesis and, consequently, these disorders. Currently, many known tyrosinase inhibitors are characterized, both natural and synthetic, but there is a great need and commercial interest in the discovery of new and better inhibitors.

The present study presents an *in silico* analysis, using molecular docking, in which a Tyrosinase three-dimensional structure (3NQ1), obtained from the Protein Data Bank database, was prepared and docked, using "AutoDock Vina" software, against a series of control compounds, in order to validate the structure. After validation, a library of 37 naturally derived flavonoids were also docked using the same structure and the same docking software, and the predicted free energy of binding and docked conformation were analysed. Three compounds presented the lowest energy of binding: myricetin (Table 3), fisetin and syringetin, with values of -8.3, -8.3 and -8.1 Kcal/mol, respectively. Although these results are promising, experimental validation will be necessary.

Introduction

Tyrosinase (EC 1.14.18.1) is an enzyme that is included in the melanin biosynthetic pathway, being one of the principal enzymes responsible for catalyzing several stages in its production. The melanin synthesis process is called melanogenesis and is carried out in specialized cells called melanocytes, which in Homo sapiens are present in the epidermis, hair and iris of the eye, giving color to these structures (Cichorek et al. 2013). It is of common and scientific knowledge that melanin is one of the most important sun protection factors, since it is essential in combating radiation (Brenner and Hearing 2007), however, despite its defensive activity, several dermatological disorders are related to an increase in the number of melanocyte cells and consequently hyperpigmentation. These dermatological disorders can range from melanomas (skin cancer), to simple hyper-pigments, leading to aesthetic problems. In both of these anomalous situations, there appears to be a correlation between excess melanin and elevated tyrosinase activity. Since tyrosinase activity is a limiting factor in the production of melanin in melanocytes, its inhibition is of interest in order to prevent/treat these disorders, with a potential negative impact on quality of life (No et al. 1999; Rao et al. 2013).

In order to discover new tyrosinase inhibitors, capable of being potentially useful for the cure/treatment of diseases and dermatological disorders, a set of studies and analyzes must be carried out, in order to obtain a good inhibition rate. *In silico* studies can be very important, before moving on to experimental tests, as they can mean a reduction in costs and time needed to develop a new inhibitor.

In order to carry out these studies, it is necessary to select and prepare *in silico* the structures of the compounds to be studied. This set of compounds are called virtual libraries. Once we have obtained the tyrosinase 3D structures and properly prepared the virtual compound library, we can then carry out *in silico* investigations including molecular docking studies. Thus, we can perform a "Virtual Screening" of the compound's library, in order to try to predict which compounds in the library will have the greatest potential inhibitor against tyrosinase. Molecular docking attempts to anticipate the best binding conformation between a protein and a ligand, so that a stable complex is formed. Based on a mathematical algorithm, several parameters are calculated, and a value is calculated, the "score", which assesses the different possible configurations. This study can help to identify potential inhibitors and provide important information about the chemical component that involves these processes. For example: analyzing the structure of the protein and the location of its active center, which atoms have greater interaction with the compounds that bind, and which amino acids have greater action in stabilizing the interaction between the compounds and the protein.

Molecular Docking

Choosing 3D tyrosinase structures

The search for these structures is carried out in the PDB database. The selection of structures is essential because the more accurate and closer to the real structure, the greater the veracity and quality of the results of *in silico* studies. So, reflecting on the parameters for choosing the best structures, for the specific case of tyrosinase, and using the PDB database, it was possible to select the structures referred to in Table 1. From the analysis of this table, it is concluded that there is not a wide variety of structures available and the organisms from which they originate belong to the bacterial kingdom, which is not ideal. However, structurally, the catalytic center is usually quite conserved when comparing 3D structure between species. Thus, the use of 3D structures of bacterial tyrosinase, not being ideal, can be considered a good approximation. The 3NQ1 tyrosinase structure was then chosen because of its adequate resolution and because it co-crystallized with kojic acid, a natural tyrosinase inhibitor, commonly used in the literature as a positive control for tyrosinase inhibition studies.

Table 1 - 3D tyrosinase structures. Source: Protein Data Bank (PDB)

PDB Name	Method	Resolution	Ligand (PDB Name)	Organism	Reference
6EI4	Difração de Raio-X	2.0 Å	B5N	Bacillus megaterium	Ferro et al. 2018
6QXD	Difração de Raio-X	2.32 Å	JKB	Bacillus megaterium	Ielo et al. 2019
5OAE	Difração de Raio-X	2.7 Å	SVF	Bacillus megaterium	Ferro et al. 2018
3NQ1	Difração de Raio-X	2.3 Å	KOJ	Bacillus megaterium	Sendovski et al. 2011
5ZRE	Difração de Raio-X	2.5 Å	…	Burkholderia thailandensis	Son et al. 2018

Software Operation

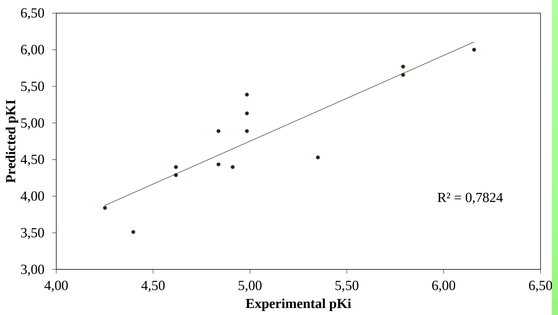
AutoDock Vina uses a search algorithm that tries to establish the best binding conformations between the compounds in a library and the chosen 3D tyrosinase structure. After completing the docking study, VINA calculates a predicted ΔG (Gibbs free energy) value, which tries to estimate how strong the bond is between them and, therefore, whether there is a potential inhibition or not. From the predicted ΔG, it is possible to calculate mathematically the predicted Ki (inhibition constant) and, from the predicted Ki value, the logarithmic form pKi is calculated. In general, the lower the predicted ΔG value, and the higher the predicted pKi value, the stronger the prediction of the bond between the compound and the protein.

Small library of control compounds

A small library of known tyrosinase inhibitors has been established, with experimental inhibition values published in the literature. This library served to control the study, thus analyzing whether the chosen structure and the software used (AutoDock Vina) are adequate to carry out the study in question. By analyzing table 2 it is possible to observe that the predicted pKi values, using VINA, are comparable with the experimental pKi values. To better understand the quality of the correlation between the experimental pKi values and the predicted pKi values, these are presented in the form of a graph that relates the two sets of values, having been calculated the correlation coefficient (R^2) between the two sets of values (Graph 1). It was found that these was a good correlation with a R^2 of 0.7824. For this type of docking studies, R^2 values of this order of magnitude demonstrate that the prediction of tyrosinase inhibition by these compounds presented great accuracy. With this study, we validated this *in silico* methodology, using the VINA software as a docking tool, and the 3NQ1 structure as the target structure of tyrosinase, to be used to try to predict the inhibition potential of other compounds of interest.

Table 2 – Molecular docking results of a library of compounds with the tyrosinase structure PDB:3NQ1. Legend: ΔG - Gibbs free energy; predicted pKi - Logarithmic form of the predicted inhibition constant; experimental pKi - Logarithmic form of the inhibition constant obtained experimentally

Compound	Predicted ΔG	Predicted pKI	Experimental pKi
Ácido arjunólico	-7,9	6,16	6,00
Ácido maslínico	-7,9	5,79	5,77
N-(2,4-dihydroxybenzyl)-3,5-dihydroxybenzamide	7,9	5,79	5,66
Morina	-7,3	5,35	4.53
Kurarinona	-6,8	4,98	5,39
Sophoraflavanone G	-6,8	4,98	5,13
Cefodizima	-6,8	4,98	4,89
Deoxyarbutin	-6,7	4,91	4,40
L-Mimosina	-6,6	4,84	4,43
Ácido betulínico	-6,6	4,84	4,89
L-Dopa	-6,3	4,62	4,29
Embelato de Potassio	-6,3	4,62	4,40
Embelin	-6,0	4,40	3,51
Ácido kójico	-5,8	4,25	3,84



Graph 1 - Correlation established between predicted pKi and experimental pKi

Aplication on a library of 37 flavonoid compounds

A molecular docking study was carried out between protein tyrosinase using the 3NQ1 structure and a library of 37 flavonoid compounds (Table 3), using VINA. The compounds were numbered from 1 to 37 to facilitate the process, both in the study and in the subsequent analysis of the results. It was then found that the 3 best results for this study correspond to compounds Myricetin (13), Fisetin (26) and Syringetin (32), with predicted ΔG values of -8.3, -8.3, -8.2, respectively. The results obtained are good, however experimental validation is always necessary.

With this study it is possible to verify that these 3 compounds have a good inhibitory potential of tyrosinase, and their analysis can be further investigated by performing other types of tests, namely molecular dynamics tests and a more detailed study on the interactions between the active center of tyrosinase and the molecules that make up each of the compounds. In figure 1 Pymol software was used to analyze the predicted 3D conformation of the docking conformation between the molecule that obtained the best result (Myricetin) and the structure of tyrosinase 3NQ1. In this way it is possible to examine which occupation is predicted by the compounds in the catalytic zone, which are the most important intermolecular bonds, and which are the most important amino acids in the predicted interaction.

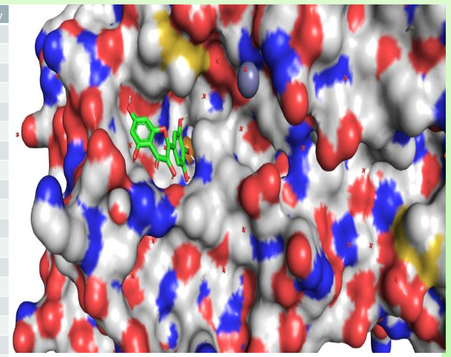


Figure 1 - Predicted biding conformation obtained by molecular docking of the tyrosinase 3NQ1 structure with myricetin. Tyrosinase structure presented in surface format and myricetin structure showing the atoms and bonds between the atoms. Image prepared in PyMOL software.

Ligand	Energy
13	-8.3
26	-8.3
32	-8,2
3	-8.2
19	-8.1
25	-8.1
14	-8.0
16	-8.0
23	-8.0
18	-7.8
4	-7.7
36	-7.6
1	-7.5
24	-7.5
27	-7.5
28	-7.5
7	-7.5
17	-7.4
6	-7.4
20	-7.3
21	-7.3
30	-7.3
5	-7.3
22	-7.2
29	-7.2
37	-7.1
9	-7.1
11	-7.0
12	-7.0
15	-7.0
35	-7.0
2	-6.9
33	-6.9
31	-6.8
10	-6.2
34	-6.2
8	-6.0

Table 3 - Library of 37 flavonoid compounds and their best ΔG value obtained in the study of "molecular docking" with the 3NQ1 structure of the tyrosinase protein

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DEVELOPMENT OF A COSMOS SOLID EMULSION

Fernanda F. Possamai[1], Ricardo Spencer[2], Susana M. Cardoso[3], Maria João Sousa[1,4], Olívia R. Pereira[4*]

[1]School of Agriculture, Polytechnic Institute of Bragança, Campus of Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal; fernandafpossamai@gmail.com; [2]UNIBIO, Lda, Cosmetics Research Department, Av. 1º de Dezembro nº41, Fracção i, 2715-109 Pêro Pinheiro, Portugal; [3]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; susanacardoso@ua.pt; [4]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; oliviapereira@ipb.pt; joaos@ipb.pt; *Correspondence: oliviapereira@ipb.pt; Tel.: +351-273-330-950

Abstract

Natural products are becoming a trend in the cosmetic market, due to a growing awareness and concern with the origin of these products. The certification of natural products, especially organic ones under the COSMOS signature preserves the integrity of the constituents, in an environmentally appropriate and safe for human health, expanding the concept of "green chemistry", guaranteeing the origin, and processing of the products, storage, manufacturing, packaging, etc. [1]. The objective of this study was to develop a moisturizing cosmetic emulsion according to COSMOS certification, in a new concept of solid cosmetic format. 59 different tests were carried out to optimise this emulsion, included variations in components and their amounts, following be evaluation of sensory aspects for each formulation. The final formulation contains components of Portuguese origin such as grape seeds oil from Douro Valley, olive oil and extract of by-products of acorn (*Quercus Ilex* L.) and essential oils of mandarin, geranium, coriander and cinnamon. The formulation has undergone accelerated stability tests with temperatures (40 °C /-12 °C). The pH, density and organoleptic characteristics were evaluate. The phenolic profile of acorn by-product (*Quercus ilex* L.) was performe by UHPLC-DAD-ESI-MS2. A questionnaire was applying to evaluate the acceptance after use of the moisturizing cosmetic emulsion. In stability tests, overall the formulation showed small color variations and less aroma intensity, maintaining hydration and solid state. The pH changes were from 4.68 ± 0.006 to 4.78 ± 0.05 and the density was maintaining at 0.73 kg/m^3. The polar extract of acorn by-product have as major compounds trigalloyl-HHDP-glucose, valoneic acid dilactone and gallic acid, known as antioxidants compounds. [2,3]. The essential oils bring beneficial properties to the skin and the aroma. In the post use of solid emulsion survey applied shown that "aroma" was the second point most valorised (47.6%), after "hydration" (90.5%). The results shown that the majority of participants (81.0%) were "very satisfied" or "satisfied" with the solid emulsion indicating the intention to use it if it is on the market (76.2%). This study highlights the research in reformulations from liquid to solid products, and the potential of using Portuguese raw materials such as acorn by-product (*Quercus ilex* L.).

Introduction

Our skin, also referred as cutaneous membrane, is our biggest organ and it is a very complex one. In its composition you can find two main layers: the dermis (the internal layer) and the epidermis (the external layer). In cosmetics, the epidermis is the primary target of skin-care products to enhance its complexion. This outer layer is constituted by two main cell groups: keratinocytes and dendritic cells and is known to be a stratified and scaled layer. In it we can find the Stratum Corneum (CS) responsible for protection functions such as substance transference between the skin's outside and inside and is considered the life barrier due to its restrict permeability and osmotic impermeability. Hydration is dependent on the CS hygroscopic properties and environmental humidity, as well as proportional to the Natural Hydration Factor (NHF) of the CS, usually lower on dry skins. Figure I shows the Corneocytes structure on a dehydrated skin, with a higher fissure count, which restraints the skin natural barrier functions. Lower hydration levels are usually associated with a fragile CS.

Figure I: Corneocytes structure on dehydrated skin (Adaptation from Matos, 2019)

Besides restraining the skin barrier functions, the hydration levels influence the CS enzymatic activity, therefore having an impact on the descaling process (the keratinocytes transformation process into corneocytes and dead cells) and the natural hydration level.

Cosmetic hydration creams are used for dry skin syndrome treatment, in order to stabilize the skin's mildness, being also capable of prevent and repair defects on the skin barrier function. LODÉN, M. (2015). Usually, these products are emulsions of heterogenic systems of emulsifying agents' immiscible phases. The COSMOS natural and organic certification main objective is to uphold a higher standard of origin tracking and sustainability of the certified products.. Figure II illustrates the COSMOS certification seals.

Figure II: COSMOS natural and organic certification signatures; Certification seals, accessed at 05/01/2020

Materials

The development of the formulation took place in the work and industrial environment of a Portuguese company of biological cosmetics that has a range of biological and preferentially portuguese compounds, from emulsifying, emolying agents, conscious agents and many other compounds. parting for practices through the basic formulation provided and described in table 1.

Table 1: Base formulation (T1.1)

TEST T1.1	Components	%	TEST T1.1	Components	%
Oily phase	Lecithin	27,37%	Water phase	Glicerine	76,92%
	Karite butter	11,39%		Sodium PCA	54,55%
	Olive oil	2,01%		Water	97,83%
	Stearic acid	86,96%			
	Cetearyl alcohool	100,00%	Cold phase	Tocopherol	99,91%
	Isoamyl laurate	60,00%			
	Cocoa butter	50,00%		Blooming Summer EO (*)	93,75%
	Broccoli oil	17,25%			
	Fractionated coconut oil	19,66%			

(*) Blooming summer EO is a mixture of essential oils produced and marketed by the company where the work was developed.

Methodology

The starting point was the base formula (Table 1), supplied by the company, with distinction of the initial compounds with solid agents, such as cetearyl alcohol and stearic acid, separated by their respective phases (aqueous, oily and cold), with the objective of obtaining a solid phase for the hydration emulsion. The oily phase components were heated to 65°C - 75°C and after being fused together, the aqueous phase was added (50 - 60°C) initiating the homogenization process on a food processor at various speeds (150-300rpm) until the mix was at 50°C (± 5°C) temperature. Then the cold phase (thermosensible compounds) is incorporated via agitation for aprox. 5 minutes so the product can be transferred to silicone molds to rest for 24 hours.

- Sensory Tests

The sensorial characteristics measured were stiffness (A), crumbling (B), oiliness (C), skin absorption (D), spreadability (E) and, solid product residues (F) using two different scales. The first being from 0 (nullable or irrelevant) to 5 (high processing) and the second scale representing the formulation perfection degree with higher amplitude, ranging from 0 (nullable or irrelevant) to 10 (high processing)

- Stability Tests

The final product was analyzed for its organoleptic characteristics (color, odor, phase separation, texture and consistency), pH and density, before and after cycles with extreme thermal variations, remaining 24 hours in freezing at -12C and, 24 hours in a bath -water at 40°C (6 cycles for each thermal variation).

- Extraction of phenolic compounds from acorn by-products

The sample of by-products of the acorn, made available in fine and dry granules, comes from the holm oak plant of the species Quercus ilex L., constituted fundamentally by skins and fruit casing, and there may be remains of the fruit itself, traces of leaves and stems of the plant. The extraction of the phenolic compounds was carried out according to the method described by Ferreira et al. with adaptations. It was then reduced to fine powder and 5 g were extracted for 15 min using a 1:20 80% hydroalcoholic solution (5 g in 100 ml of water). The extract was filtered and the residue re-extracted two more times. The total filtrate (300 ml) was concentrated using a rotary evaporator at 37 ° C. (BUCHI Labortechnik AG, Flawil, Switzerland). The resulting fraction was frozen, lyophilized and kept under vacuum in a desiccator, in the dark, for subsequent use.

- Phenolic Compounds Identification

The phenolic profile of the polar extract of the acorn by-product (10 mg / mL) was determined by analysis in liquid chromatography (UHPLC-DAD-ESI-MS2) using an apparatus equipped with an Ultimate 3000 diode detector (Dionex Co., San Jose , CA, USA) and a Thermo LTQ XL mass spectrometer (Thermo Scientific, San Jose, CA, USA), following the method described by Afonso et al.42 Phenolic compounds were identified using standard commercial compounds whenever possible, or else , based on the interpretation of ultraviolet (UV) and mass spectrometry (MS and MS / MS) data, in addition to comparison with the literature.

- Questionnaire for the characterization of the skin's moisturizing emulsion after use

In order to assess the after use perception of the solid moisturizing cream to evaluate the points of greatest satisfaction and dissatisfaction of the volunteers and to understand the potential for acceptance of the developed solid cream, a questionnaire was applied online, with a sample of 21 volunteers, after daily use of the product for a period of 5 days, following the use instructions provided in an explanatory leaflet.

Results

The first formulation stage was based on the development of a stable solid base, that is, that the emulsion had a good texture, solidifying after the total homogenization of the compounds and cooling of the formulation, maintaining a good appearance and that it was moisturizing.The results of the tests developed in this first stage are described in table 3.

Table 3: test sensory parameters (0 to 5 scale).

TEST	A	B	C	D	E	F
T2	3	4	4	3	1	4
T2.2	3	5	3	3	1	5
T3.1	4	2	1	5	5	1
T3.2*	4	4	1	5	5	1
T4	4	0	0	2	3	1
T5	4	1	0	2	3	2
T6	3	5	0	2	3	5
T7.1	4	0	0	3	4	0
T7.2*	4	1	0	3	4	1
T8	4	0	0	3	3 – 4	0
T9	4	1	0	2	3	1
T10	4	0	5	3	5	0
T11	5	0	0	0 - 1	1	0
T12	5	0	0	0 - 1	1	0
T13	4	0	3	4	4	0
T14	4	0	2	4	4	0
T15	4	0	4	3	5	0
T16	4	0	4	3	5	0
T17.1	4	0	5	4	5	0
T17.2	4	0	5	5	5	0
T17.3	4	0	5	4	5	0
T17.4	4	0	5	4	5	0
T18.1	4	0	3	4	4	0
T18.2	4	0	2	5	4	0
T19	4	0	2	4	4	0
T20	4	0	1 - 2	4	4	0
T21	4	1	1 - 2	4	4	0
T22	4	0	1	4	4	0
T23	4	0	1	4	4	0
T24	4	0	2	5	4	0
T25	4	0	2	4	4	*
T26	3	1	2	4	4	2
T27	4	0	2	5	4	0
T28	2	1	2	4	5	2
T29	2	1	2	4	4	2
T30	2	1	2	4	4	2
T31	3	1	2	4	4	1
T32	4	2 – 3	2	4	4	1
T33	2	1	2	4	5	0
T34	2	1	1 - 2	4	5	0
T35	4	0	2	5	4	0
T36	3	1	1	4	5	1
T37	2 – 3	4	1	4	5	4
T38	3 – 4	1	2	5	5	0
T39	4	0	1 - 2	5	3	0
T40	0	na	na	na	na	na
T41	4	0	2	5	4	0

As can be seen, the first tests all presented some degree of solid residue, indicating a low rigidity of the product. As advances this degree has been reduced, in order to achieve a high degree of rigidity with no product residue. Tests were carried out with incorporation of starch in cassava starch, T2.2 and T3.2, 10% and 5% respectively, observing a high degree of crumbling in both final products, while the sample T2.1 and T3.1 (without starch) showed a lower degree of crumbling, but present, indicating that more compounds should be tested in the formulation.

The water had been added in different proportions and ways during the tests, either in its pure form or in lavender hydrolate, or even by the aqueous extract of acorn (Quercus ilex L.) and, even in the case of a solid emulsion , proved to be fundamental in the formulation, since the samples where water was not present, T13, T14, T15 and T16, there was less effectiveness of the emulsification process and, after application on the skin, a hydration effect was noted lower than desired, with a slight excess of oil, according to the results observed in table 3.

After achieving the best result for all aspects, as evidenced in the T41 test, a second step to improve the tests was carried out, as shown in table 4. In samples T45 and T46, two compounds stand out, the Olive squalene wax (T45) and Squalene-based olive wax butter (T46), which were incorporated in place of carnauba wax. (of stiffening properties). The results observed in the T45, were very promising and, the slightly high oiliness (grade 6) is of interest for a moisturizing emulsion. It is very stable and much easier to work with due to its melting point being lower than carnauba wax (the main hardener used until then), in addition to protecting the skin and preventing moisture loss, being also used in anti creams. -aging. [5]

The best result obtained was that of the T52.1 test, which obtained an average scale value (5) for all sensory parameters, with no degree of crumbling or product residues, therefore originating the final formulation of the present work.

Table 4: Sensory parameters (0 to 10 scale).

TEST	A	B	C	D	E	F
T41	5	-	6	4	5	-
T42	3	+	6	na	2	+
T43	5	-	5	5	5	-
T44	5	-	5	5	5	-
T45	5	-	6	6	6	-
T46	5	-	7-8	6	7	-
T47	5	-	5	5	5	-
T48	5	-	6-7	5	5	-
T49	3	+	7-8	4	6	+
T50	3	+	7-8	4	6	+
T51	3	+	7	4	6	+
T52.1	5	-	5	5	5	-
T52.2	5	-	5-6	5	5	-
T53	5	-	6-7	5	5	-

+: presence
-: absense

Stability Tests

After the completion of the cycles of stability tests, the sample solidified again (after 24h) maintaining the rigidity characteristics of the solid emulsion, however its color was slightly lighter, as shown in Figure 1. The original aroma remained, but with less intensity. The pH changes a little from 4.68 ± 0.006 to 4.78 ± 0.05 but the density remained the same after all the stability tests, 0.73 kg / m3.

Identification of phenolic compounds from acorn by-products (Quercus ilex L.)

The qualitative results of the UHPLC-DAD-ESI-MS2 analysis of the polar extract of the acorn by-product (Quercus ilex) are illustrated in Figure 2, referring to the chromatogram obtained at 280 nm. Highlighting the majority compounds in the sample, Trigaloil-HHDP-glucose (peak 13), Valoneic acid dilactone (peaks 14) and gallic acid (peak 3).

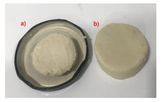

Figure 1: (a) sample after 6 cycles of accelerated stability tests; (b) control sample.

Characterization of the after use of the moisturizing emulsion for the skin

Containing essential oils of mandarin, geranium, coriander and cinnamon seeds, which bring a touch of velvety freshness to the aroma, an aroma that proved to be the second point that most pleased the participants (47.6%) in a post survey use of "solid cream", second only to the hydration effect that receives 90.5% acceptance. The development and evaluation of the solid emulsion by a small sample of 21 individuals, revealed an excellent adherence and satisfaction to this product rich in fatty acids and special compounds, such as the extract of the acorn by-product

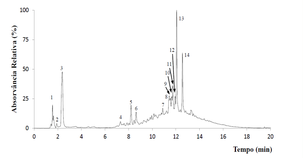

Figure 2: Chromatographic representation of the hydroalcoholic extract of the acorn by-product (Quercus ilex L.) at 280 nm. The numbers in the figure correspond to the UHPLC-DAD-ESI-MSn peaks.

Conclusion

During the development of this study, it was possible to verify in different ways the interactions of the emulsification process and the emulsifying and co-emulsifying agents in a cosmetic emulsion, especially when we change the proposal from consistency to solid.

Most of the emulsifiers available on the market are O / A type, when deciding to develop a solid emulsion the number of fats and lipophilic compounds increased and the search for an A / O emulsifier was necessary, however because it is also a biological cosmetic emulsion, subject to COSMOS certification, the number of emulsifiers available for testing are even more restrict, which made the development of the project challenging and engaging.

Water, whether pure or in the acorn extract itself, proved to be fundamental in the formulation, even though it is a solid emulsion, with a greater effectiveness of the emulsification process and a greater hydration effect, similar to the hydrating creams available on the market , where, for the most part, water is the main ingredient.

As for the tests of accelerated stability, the relative density of the solid emulsion remained at 0.73 Kg / cm3, there was no relevant pH variation, although the color of the sample has changed to a slightly lighter tone. As for the reduction of the intensity of the aroma, it was already foreseen, since the essential oils are thermosensitive compounds and, therefore, easily volatile with sudden temperature fluctuations.

Most of the sample participants who evaluated the solid moisturizing cream developed in this study, after using it, classify their skin as normal (42.90%) or dry (33.90%), which may justify the demand for more oils and greater adhesion to this solid emulsion rich in fatty acids.

The extract of the acorn by-product (Quercus ilex L.) revealed a great commercial potential for the cosmetic industry, especially biological, since it is a by-product and therefore, the reuse of a waste is essential in reducing the environmental impact, and to grow awareness for a deeper academic interest in the investigation and qualification of this by-product.

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Acknowledgements

Thanks to FCT (Portugal) and the ERDF under the PT2020 Program for financial support to CIMO (UIDB/00690/2020) and LAQV-REQUIMTE (UIDB/50006/2020). SMC acknowledges the research contract (project AgroForWealth, CENTRO-01-0145-FEDER-000001).

DETERMINATION OF THE TOTAL ANTIOXIDANT ACTIVITY OF THE TOASTED COFFEE BY-PRODUCT

Fernanda Maria da Silva, Soraia Ferreira Bezerra, Emannuel Ítalo Alves Campos, Nathalia Ferreira Souza, Nathalia Santos Araújo & Edemilson Cardoso da Conceição

Faculdade de Farmácia, Universidade Federal de Goiás (UFG)

INTRODUCTION

Coffea sp. (coffee tree) is a shrub of the Rubiaceae family of great interest worldwide due to the presence of caffeine in its beans, which has a stimulating property, and for presenting a pleasant aroma and flavor. The habit of consuming toasted coffee ends up generating a large amount of by-products which are dispersed in the environment and, consequently, contributing to the environmental impact. Therefore, there is a need for in vitro studies that can prove the presence of beneficial chemical properties, such as antioxidants, in agro-industrial waste to develop new sustainable products.

OBJECTIVE

The objective of this work was to evaluate the total antioxidant activity of the toasted coffee by-product.

Image I - By-product of toasted coffee.

MATERIALS

It was acquired the toasted coffee by-product

↓

Subjected to dehydration in a forced circulation oven and air renewal at 60 ° C

↓

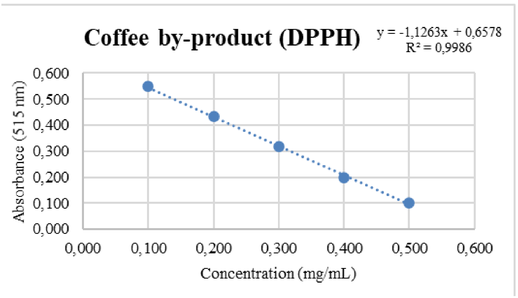
The dried material was kept away from light and moisture

METHODOLOGY

The total antioxidant activity by capturing the free radical DPPH (2,2-diphenyl-1-picryl-hydrazil) was determined according to the method described by Brand-Williams, Cuvelier and Berset (1995), with adaptations.

RESULTS

The result found in this current study (IC50 of 277 µg / mL) showed that the by-product of toasted coffee has bioactive compounds with antioxidant potential that were responsible for the reduction of absorbances, at a wavelength of 515 nm, from capture of the DPPH free radical (Figure 1).



CONCLUSION

✓ The by-product of toasted coffee contains secondary metabolites with antioxidant properties, such as phenolic compounds, which were able to capture the free radical DPPH and promote its stability.

RECOMMENDATIONS

✓ Therefore, it is suggested to continue the study from other antioxidant tests to fully characterize the compounds that neutralize or reduce the free radical and promote the application of the by-product in technological innovation in the pharmaceutical, cosmetics and food industries.

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ACKNOWLEDGEMENTS

PDI Bioprodutos

CAPES

UFG

Water-in-oil emulsions based on natural ingredients for functional applications

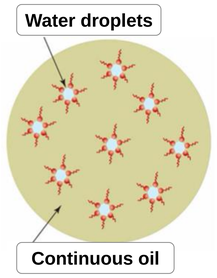
Giovana Colucci[1], Isabel Patrícia Fernandes[1*], Filomena Barreiro[1]

Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Campus Santa Apolónia - 5300-253 Bragança, Portugal

(*ipmf@ipb.pt)

Indroduction

Water-in-oil (W/O) emulsions are biphasic delivery systems that comprise water droplets dispersed into a continuous oil phase [1]. This type of emulsion is widely applied in moisturizing cream formulations because it promotes long-lasting adherence to the skin and improved water resistance. The development of natural-based W/O emulsions is a topic of great interest for the cosmetic industry due to their high biological activity value and environmental-friendly aspects [2].



Green tea extracts are known to have healthy properties, such as antioxidant and antimicrobial activities, due to the presence of catechins, among other functional groups [3]. The sweet almond oil is rich in oleic acid, having moisturizing, softening and nutritive properties, being widely used in the cosmetic industry [4].

In this context, the present work aimed to develop a stable W/O emulsion based on sweet almond oil and green tea aqueous extract for a moisturizing product with antimicrobial and antioxidant properties.

Experimental Procedure

Chemical System

- *Camellia sinensis* aqueous extract (Essencia D'Um Segredo, Portugal)
- Sweet almond oil (LabChem, Portugal)
- Span 80 (AlfaAesar, Germany)
- Tween 80 (Pancreac Applichem, Spain)
- Distilled water

Emulsions Preparation and Characterization

Samples of a base emulsion and emulsions with green tea extract at a content of 3.75% and 5% (w/v, water base) were prepared. The used W/O ratio and emulsifier system were 40/60 and a Span 80/Tween 80 mixture (54/46 ratio, v/v), respectively. The samples were produced by firstly preparing a coarse emulsion using a mechanical homogenizer (11000 rpm, 5 min), being followed by a droplet size reduction stage, using a high-pressure homogenizer (HPH) during 12 cycles (Figure 1).

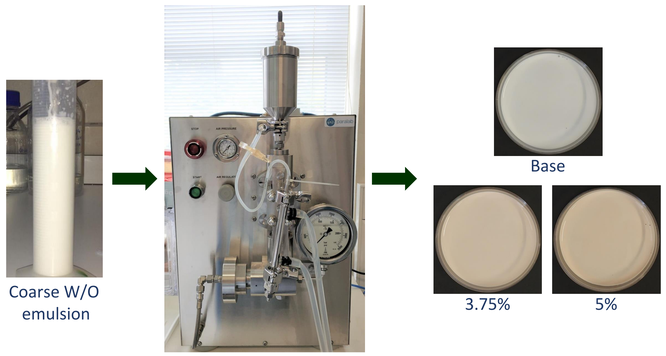


Figure 1. Emulsion's preparation procedure.

The emulsions were characterized through the evaluation of the antioxidant activity (DPPH radical-scavenging activity method) and antimicrobial activity (agar diffusion test using *S. aureus* ATCC 29213 and *E. coli* ATCC 25922). The formulation stability was accessed by visual inspection and by optical microscopy along the storage time (3 months) at 4 ºC.

Results

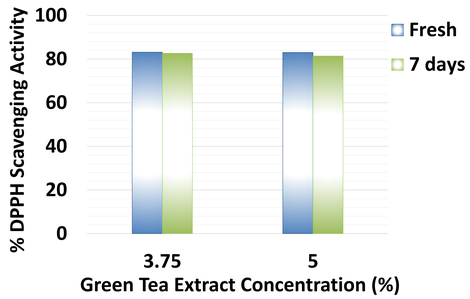


Figure 2. Antioxidant results of the fresh emulsions and after 7 days of storage.

Antioxidant Analysis

The emulsions containing green tea extract showed a DPPH scavenging activity of approximately 83%, being independent of the used extract concentration. A similar result (82%) was obtained after 7 days of storage (Figure 2). For the base emulsion, no antioxidant activity was detected, indicating that this property is related to the green tea presence in the formulations.

Antimicrobial Analysis

The emulsions with 3.75% and 5% green tea had a prolonged effect against *S. aureus* bacteria, maintaining the same inhibition zone (10 and 12 mm, respectively) after 24 (Figure 3) and 96 hours of incubation. For *E. coli* no inhibition was detected, while the base emulsion did not present any activity due to the absence of the green tea extract.

Stability Analysis

The emulsions were macroscopic and microscopic stable with no instability phenomena for, at least, 3 months of storage (Figure 4).

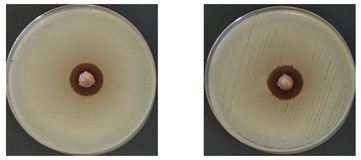


Figure 3. Antimicrobial assay for emulsions against *S. aureus* after 24 hours of incubation.

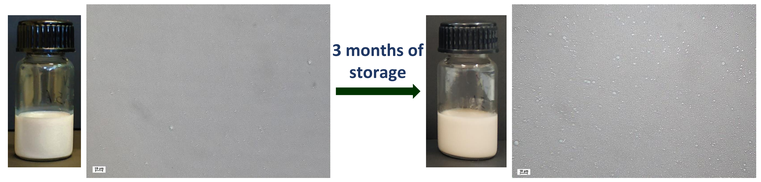


Figure 4. Visual and microscopy images of 5% green tea emulsion.

Conclusions

In this work, stable W/O emulsions functionalized with green tea extract were prepared. The extract presence imparted antimicrobial and antioxidant properties to the formulations, making them of great interest for application in areas such as cosmetics. However, a more extensive characterization is still required, in order to improve the knowledge on the emulsions' physicochemical properties.

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Acknowledgements

Base Funding - (UIDB/00690/2020) of CIMO funded by national funds through FCT/MCTES (PIDDAC). National funding by FCT, through the institutional scientific employment program-contract with I.P. Fernandes.

OLIVE-OIL BY PRODUCTS: IMPACT IN COSMETIC FORMULATIONS

Andreia Nunes[1], Joana Marto[1], Lídia Gonçalves[1], Carmo Fraga[2], Helena Margarida Ribeiro[1]

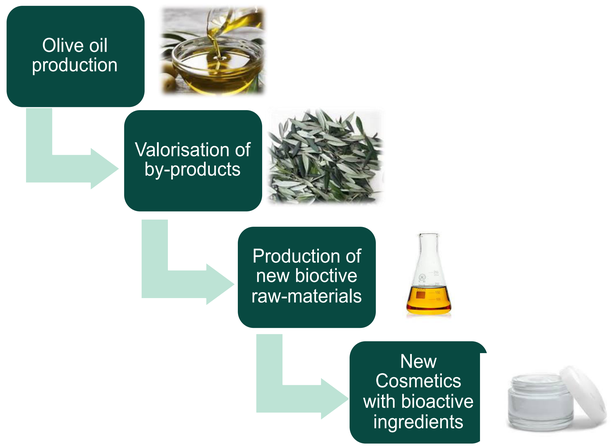
1 - Research Institute for Medicines – iMed.ULisboa, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

[2] - Sovena Portugal – Consumer Goods, S.A., com sede em Rua Dr. António Borges nº2, Edifício Arquiparque 2 – 3º andar, 1495-131 Algés.

* ✉ **hribeiro@campus.ul.pt**

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

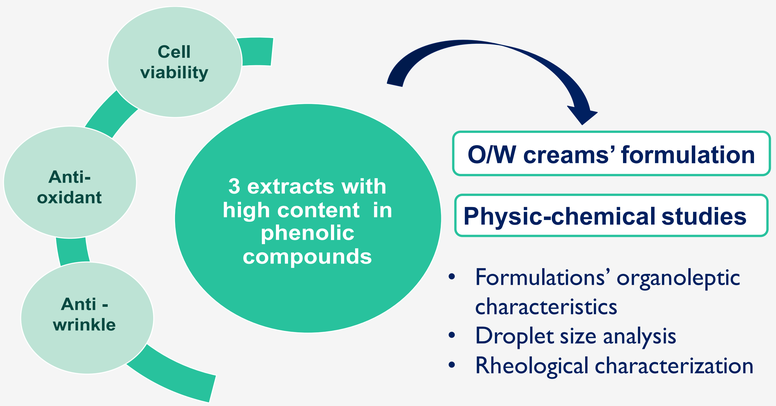
Introduction



Aim

Evaluation of three olive oil by-products extracts', obtained without using organic solvents, *in vitro* bioactivities and their impact and characterization in oil-in-water (O/W) creams for cosmetics.

Methods



Conclusion

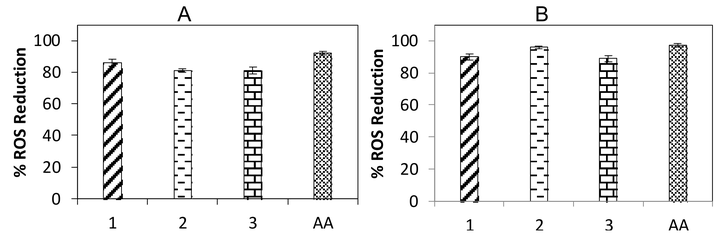
The *in vitro* methods demonstrated that olive by-products presented antioxidant and HNE activities. Furthermore, these olive oil by-products extracts present an impact in cosmetic formulations' appearance, pH and rheological performance being suitable for cosmetic use.

Results and Discussion

Enzymatic activity

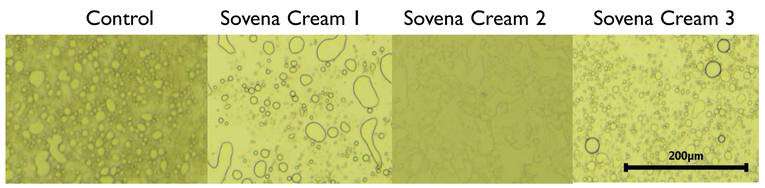
Oive oil by-products extracts	Enzymatic inhibition (%)			
	Elastase	Collagenase	Hyaluronidase	Tyrosinase
1	94 ± 2	0 ± 5	0 ± 13	10 ± 4
2	97 ± 0	19 ± 7	31 ± 10	13 ± 15
3	100 ± 0	9 ± 4	20 ± 16	16 ± 6

Antioxidant Capacity



All extracts present a highly favorable ROS reduction in HaCaT cells, being in the same reduction range as ascorbic acid ((A) H_2O_2 solution; (B) UVB light for 15min.) and inhibitory ativity against elastase. Nevertheless, they did not show high *in vitro* tyrosinase, collagenase and hyaluronidase inhibition.

Droplet size



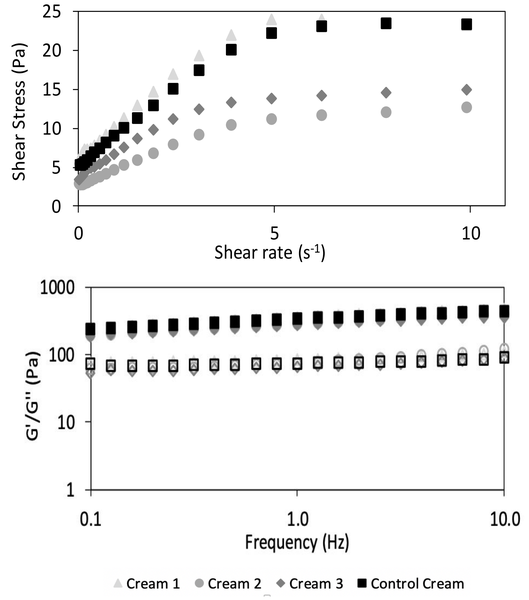
In ordinary light, all creams contained distorted oil droplets.
The control cream contained smaller droplets than cream 1, whereas droplet sizes were largest in the creams containing extracts 2 and 3.

Physio-chemical studies

	O/W emulsions			
	Sovena Cream 1	Sovena Cream 2	Sovena Cream 3	Sovena Control Cream
Organoleptic characteristics	Beige and opaque appearance with extract's characteristic smell			White and opaque appearance
pH	4.74	5.05	4.50	5.54
Apparent viscosity (Pa.s) (at 0.1s^{-1})	59.76	28.45	34.08	52.97
Droplet size d(50) (µm)	34.98	44.34	43.55	16.33

Creams respect the skin's natural pH, thereby all the formulations should present an acidic-neutral pH value . The average droplet size for these formulations is higher than the Control Cream (d (50) = 16µm), which may be related to the incorporation of extracts in the formulation.

Rheological studies



All formulations present shear thinning behavior.
The storage modules (G') were higher than their loss modules (G''), with a predominating "solid-like" behavior, revealing viscoelasticity.

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This work was supported by Sovena Portugal Consumer Goods through the project Oil4Health: From Olive To Health LISBOA-01-0247-FEDER-038554, funded by the Portugal 2020 program – Programa Operacional Regional de Lisboa, and FCT Portugal (UIDB/04138/2020 to iMedUlisboa and CEECINST/00145/2018 to J. Marto).

Substantiation Of Hibiscus Extract Use In Cosmetics

Inga Iždonaitė-Medžiūnienė (PhD)
University of Applied Social Sciences (SMK), Klaipėda, Lithuania

Abstract

The conducted research analysis and synthesis allow to prove a huge potential of hibiscus extract use in cosmetics for skincare.

The paper highlights the benefits of hibiscus extract and presents areas of its positive effects.

Key words: hibiscus extract, hibiscus extract use in cosmetics, benefits of hibiscus extract

Introduction

In the Indian Ayurvedic literature, different parts of the hibiscus plant are recommended for various ailments like hypertension, fever and liver disorders.
The plant is traditionally used:

1. *as an antiseptic, aphrodisiac, as having emollient, digestive, diuretic, brightening, soothing properties;*
2. *as a means rich in flavonoids and proanthocyanidins;*
3. *as a means with phytotherapeutic possibilities.*

Findings

Studies prove the cosmetic ***skin care potential of hibiscus extract***.
Hibiscus rosa-sinensis flower extract has been found to have ***a protective effect against the sun*** by absorbing ultraviolet radiation [6].
Antioxidant and free radical reducing effects have been demonstrated [3] [1]. The properties of Hibiscus rosa-sinensis extract, which has a high antioxidant effect, are also proved by Garg et al. in 2012.
Hibiscus plant extracts have an incredibly ***low degree of toxicity*** [1].

Hibiscus and its isolated compounds may be an important source of ***therapeutically useful*** products, given the stated nutritional and pharmacological characteristics and the relative safety of the extract [1].
As hibiscus extracts contain organic acids and minerals, they can have a direct ***relaxing effect*** on smooth muscles, and the ***antipyretic, antinociceptive and anti-inflammatory effects*** of this extract [1].

Methodology

The research is based on scientific literature ***analysis*** and ***synthesis***.

Results

The use of hibiscus flower extract as an active ingredient in cosmetic products for skin care has been observed to have the following ***effects*** [5]:

1. the ***oxidative*** effect of this active substance;
2. the ***stimulating*** effect of stimulating cellular metabolic processes.

The use of hibiscus extract ***increases the therapeutic potential*** of fibroblasts and keratinocytes [4].

Specifically, hibiscus extract ***significantly stimulates fibronectin and collagen synthesis*** by 16% and 60%, respectively, and fibroblast contraction is increased by 30%.

These results were confirmed in skin care procedures where hibiscus extract markedly ***accelerated wound healing, epithelial formation, and fibronectin production***.

Conclusions

1. The conducted research substantiates the ***positive use of hibiscus extract in cosmetics*** for its numerous advantages.
2. Studies confirm that ***hibiscus extract increased the expression of genes involved in skin hydration and homeostasis*** [4].

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BIOACTIVITY OF PLANT EXTRACTS: ASSESSMENT OF CYTOTOXICITY, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

Joana Rolo[1], Inês Almeida Pinto[1], Frederico Holtreman[1], Carlos Gaspar[1,2,3], Ana Sofia Oliveira[1,2], Rita Palmeira-de-Oliveira[1,2,3,4], José Martinez-de-Oliveira[1], José Carlos Gonçalves[5,6], Fernanda Delgado[5,6], Ana Palmeira-de-Oliveira[1,2,3]

[1]CICS-UBI- Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal; [2]FCS-UBI- School of Health Sciences, University of Beira Interior, Covilhã, Portugal; [3]Labfit-HPRD: Health Products Research and Development Lda, Covilhã, Portugal; [4]Center for Neuroscience and Cell Biology, University of Coimbra, Portugal; [5]Escola Superior Agrária do Instituto Politécnico de Castelo Branco, Portugal; [6]CERNAS: Research Center for Natural Resources, Environment and Society, Agriculture Science Research Group, Castelo Branco, Portugal.

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Abstract

The increased interest in natural cosmetic formulations has created the need to extensively characterize plant extracts. Plant extracts are known for their overall bio-compatibility, low toxicity and numerous health benefits, being used in traditional medicine over the ages. In this study, we aim to contribute to this huge body of knowledge by characterizing extracts of plants produced/endogenous in Portugal, with the ultimate goal of assessing the interest of inclusion of these extracts in cosmetic or pharmaceutical formulations. To achieve our aims, hydrolates of six different plants were obtained by hydrodestillation of aerial parts (*Matricaria chamomila, Hammamelis virginiana, Echinacea purpurea, Cistus ladanifer, Cupressus lusitanica, Thymbra capitata*). An aqueous infusion of the leaves of *Ocimum basilicum* was also studied. The antimicrobial activity against Gram-positive and Gram-negative bacteria was determined by microdilution broth assay. Antioxidant activity was assessed using the DPPH reduction assay. Cytotoxicity against skin fibroblasts (3T3) was determined using MTT assay. We found that the great majority of plant extracts had mild antibacterial activity against Gram-negative bacteria (minimum inhibitory concentration, MIC≥50%). The exceptions were *T. capitata* and *C. lusitanica* that were active against Gram-negatives (MIC 6,25% each). *E. purpurea, C. ladanifer* and *O. basilicum* hydrolates were also not very active against Gram-positives (MIC≥50%). The same was observed *for C. lusitanica* hydrolate. The remaining extracts showed activity against Gram-positive bacteria (*M. chamomila*, MIC 25%; *H. virginiana*, MIC 25%; *T. capitata* MIC 6,25%). Regarding the antioxidant activity, only two extracts showed a very strong ability to reduce DPPH: *T. capitata* and *H. virginiana*. *C. ladanifer* and O. *basilicum* showed a moderate antioxidant activity and the remaining extracts did not showed a relevant ability to reduce DPPH. Finally, only one extract was able to reduce fibroblasts' viability by 50% at a lower concentration: *T. capitata* (EC50, 18%). The remaining extracts were cytotoxic only (considering a reduction of 50% of fibroblasts' viability) at around 30% *(M. chamomila, H. virginiana, C. lusitanica*) or 50% (*C. ladanifer*, *O. basilicum*). Our results suggest that by showing, although modest, antibacterial activity, moderate antioxidant activity and low cytotoxicity *C. ladanifer* and *O. basilicum* extracts are excellent candidates to be included as ingredients in natural cosmetic formulations.

Aim

To characterize the bioactivity and the safety of selected plant extracts to be used in cosmetics and pharmaceutical formulations, with the overall additional perspective of valorization of portuguese endogeneous resources.

Matricaria chamomila
"Camomila"

Hamamelis virginiana
"Hamamélis"

Equinacea purpurea
"Equinácea"

Cistus ladanifer
"Esteva"

Cupressus lusitanica
"Cedro-do-Buçaco"

Thymbra capitata
"Tomilho"

Ocimum basilicum
"Manjericão"

inovep
Inovação com Extratos de Plantas

Methodology

Antimicrobial Activity

Microdilution assay against Gram-positive bacteria (*S. aureus* ATCC 6538) and Gram-negative bacteria (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027) [1]

Citotoxic Activity

MTT assay on a mouse fibroblast cell line (NIH/3T3; ATCC CRL-1658) [2]

Antioxidant Activity

DPPH reduction assay [3-5]

Results

Phyto-chemical analysis of plant extracts

Table1. Major component of plant extracts as determined by GC-MS analysis. The proportion of the predominant compound in each extract is also showed, as well as their chemical class.

Plant	Major component	Concentration (%)	Class
M. chamomila	α-Bisabolol oxide A	80.67	Oxanes
H. virginiana	Not performed*	Not performed*	Polyphenols, flavonoids, ionones [6]
E. purpurea	trans-Verbenol	11.70	Flavonoids
C. ladanifer	4-Hydroxy-3-methylacetophenone	21.58	Alkyl-phenylketones
C. lusitanica	Terpinen-4-ol	38.93	Monoterpenes
T. capitata	Carvacrol	98.11	Monoterpenes
O. basilicum	Linalool	38.34	Monoterpenes

* Low yield during hydodestillation process

Results

Antibacterial and cytotoxic activity

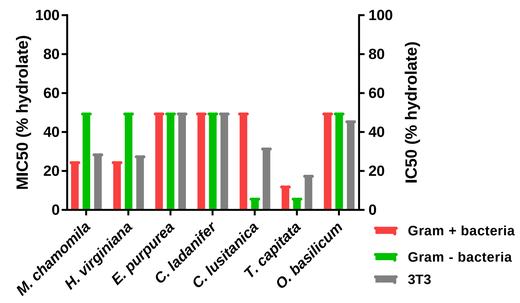

Fig.1. Ability of each extract (%,V/V) to reduce 50% of bacterial growth (Gram-positive/ Gram-negative bacteria) or 50% of 3T3 skin fibroblasts´viability. The results represente the average between at least two independente exprimenrs

Antioxidant activity

Table 2. Extract concentration (%,V/V) able to reduce half of the DPPH molecules in test. IAA: index of antioxidant activity. The antioxidant capacity based on IAA classification is also showed.

Plant	EC50 (%,V/V)	IAA	Antioxidant capacity
M. chamomila	47.55	0.001	Low
H. virginiana	0.04	7.99	Very strong
E. purpurea	36.22	0.175	Low
C. ladanifer	14.32	0.88	Moderate
C. lusitanica	38.12	0.25	Low
T. capitata	5.78	1.59	Strong
O. basilicum	12.38	0.65	Moderate

Discussion

- Each plant extract had a diferent major compound in their phytochemical composition. For three extracts (*C. lusitanica*, *T. capitata* and *O. basilicum* hydrolates) the major compound belonged to the monoterpenes chemical class (Table 1).
- The activity of the extracts against Gram-positive and Gram-negative bacteria was different. These results are probably related with the different ability of the compounds present in these extracts to destabilize bacterial wall and membranes. While *M. chamomila* and *H. virginiana* hydrolates were more active against Gram-positive bacteria, *C. lusitanica* and *T. capitata* hydrolates were more active against Gram-negative bacteria. The remaining extracts had a moderate antibacterial activity and were equally active against Gram-positive and Gram-negative bacteria (Fig. 1).
- Cytotoxicity results against skin fribroblasts revealed that only *T. capitata*, *M. chamomila*, *H. virginiana* and *C. lusitanica* extracts showed significant toxicity (Fig. 1).
- Regarding the ability of each extract to reduce bacterial and skin fibroblasts' growth, we found that the least active extracts (around 50% of the extract needed to produce an effect) *were E. purpurea*, *C. ladanifer* and *O. basilicum* hydrolates. (Fig. 1).
- The extracts with most antioxidant effect, as determined by the ability to reduce DPPH were *H. virginiana* and *T. capitata* extracts (Table 2).
- Overall, the most active extract among the studied ones was *T. capitata* hydrolate. The major compound in this extract, carvacrol, has been previously associated with its high bioactivity [7].

Conclusions

- A moderate bio-activity regarding antimicrobial, antioxidant and cytotoxic abilities is a desirable characteristic for putative cosmetic ingredients.
- By showing moderate antioxidant activity and biocompatibility with both skin microflora and skin fibroblasts, *C. ladanifer and O. basilicum* hydrolates should be given further consideration as valuable cosmetic ingredients.

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Acknowledgements

This work was supported by "INOVEP project – Innovation with Plant Extracts", I&DT projects for companies in collaboration with scientific entities, project number 33815, Centro2020 and by Foundation for Science and Technology (FCT), through funds from the State Budget, and by the European Regional Development Fund (ERDF), under the Portugal 2020 Program, through the Regional Operational Program of the Center (Centro2020), through the Project with the reference UIDB/00709/2020.

NPA
Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

IN SILICO STUDY OF A LIBRARY OF FLAVONOIDS AS POTENTIAL COLLAGENASE INHIBITORS

José Pedro Lemos Pinheiral [1], Filomena Adega[2], Rui M. V. Abreu[3]

[1]Escola de Ciências da Vida e Ambiente, Universidade de Trás-os -Montes e Alto Douro (UTAD), Quinta de Prados 5000-801, Vila Real, Portugal; [2]Departamento de Genética e Biotecnologia, Universidade de Trás-os -Montes e Alto Douro (UTAD), Vila Real, Portugal; [3] Centro de Investigação da Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia 5300-253, Bragança, Portugal. Email: jplp10399@gmail.com

Abstract

Collagenase (MMP-13) is a matrix metalloproteinase involved in the degradation of collagen, being collagen an essential constituent of human tissues like cartilage, tendons, and skin [1]. The inhibition of collagenase is a therapeutic target for several diseases such as osteoarthritis and skin age, since these conditions can be caused by an uncontrolled degradation of collagenase. Therefore, the research for natural metabolites and products that could inhibit Collagenase is ongoing.

In the present study, a total of 37 flavonoids, prepared by Pradiba *et. al* in 2018 [2] and used against metalloproteinase-9 (MMP-9), were analyzed as potential inhibitors of Collagenase. A Molecular Docking study was performed, using AutoDock Vina software, and a three-dimensional structure of Collagenase was used as protein target.

From the docking studies with Autodock Vina and posterior virtual screening through MOLA software, 4 major compounds with theoretical good potential to inhibit Collagenase were identified. Kaempferitrin presented the best Collagenase inhibition potential, with a predicted Ki (Constant Inhibition) value of 91,86 nM, followed by Nicotiflorin with a predicted Ki value of 108,75 nM, Rutin with a predicted Ki value of 128,75 nM and Broussoflavonol F with a predicted Ki value of 180,44 nM. In conclusion, the 4 highlighted flavonoids may have good potential to inhibit Collagenase, however this predicted activity must be experimentally verified.

Introduction

Collagen is the most abundant individual protein in mammalian animals, representing about 30% of total proteins. After being secreted, they are grouped into fibers responsible for the functional integrity of tissues such as bone, cartilage, skin and tendons [1].

Matrix metalloproteinase are zinc-dependent endopeptidases that are involved in the degradation of extracellular matrix proteins. Currently, 27 human MMP subfamilies are known based on the specificity of the protein substrate [3]. Collagenase (MMP-13) (EC 3.4.24.B4) belongs to one of these subfamilies which includes, in addition to collagenase, MMP-1 and MMP-13, and mainly disintegrates the collagen fibers of cartilage, skin and cancer cells.

Structurally, collagenase is characterized by a triple helix consisting of three distinct α chains. Regarding the three-dimensional structure (Fig.1), collagenase in *Homo sapiens* is a hydrolase with two subunits A and B, which have a length of about 172 amino acids each in the catalytic domain (aa 103 - aa 274). Typically associated with the protein are zinc ions, at least one of which is in the active center and binds to three histidine residues and one molecule of water and, generally, calcium and sodium ions. It consists of a catalytic domain and a hemopexin domain, both essential for collagenase to break a collagen triple helix. The two domains together form a collagen-binding zone that is exclusive to collagenases [4]. They have several binding sites, the main one being in the protein's catalytic zone (catalytic triad), which is called the "S1' pocket" [5]. The "S1' pocket", together with the catalytic zinc ion, is the most studied area of the protein with the aim of discovering potential drugs that combat the action of collagenase [6].

The benefits of discovering drugs that can inhibit the action of collagenase are innumerous in fields such as health and cosmetics.

For example, the study of collagenase inhibitors can be a good advance in the treatment of osteoarthritis, since, MMP-13 mainly catalyzes the hydrolysis of type II collagen, the main structural component of the cartilage matrix [7].

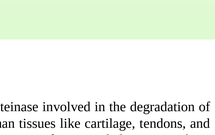

Figure 1 - 3D structure of the collagenase protein in Homo sapiens (PDB: 5B5O). Chains A and B displayed by green and cyan blue, respectively. Zinc, calcium and sodium ions represented by blue, orange and magenta spheres, respectively. Figure obtained and prepared in Pymol software (The PyMOL Molecular Graphics System, Version 2.4 Schrödinger, LLC).

On the other hand, skin aging is a complex biological process influenced by a combination of endogenous and exogenous factors, one of which is collagen degradation by collagenase, mainly induced by UVB radiation. So, inhibitors of that degradation could be a good advance in cosmetics.

Many inhibitors of collagenase, both natural and synthetic, have been studied. A large percentage of natural inhibitors have been isolated from plants, mainly polyphenols, such as catechins, and flavonoids, and cyclitols. Generally, natural collagenase inhibitors are of interest for the pharmaceutical and cosmetic industries, as they are generally cheaper compared to synthetic inhibitors, or because they have less toxicity in the first place. However, not all known naturally occurring inhibitors are effective for a variety of reasons, for example, because of the low solubility in body tissues, or because they have only reasonable inhibitory power. Therefore, there is a wide variety of synthetic compounds, such as steroids, which are structurally based on natural collagenase inhibitors.

It is common practice for the pharmaceutical industry to design and produce a set of inhibitors with an equivalent base structure varying only a few radicals, to be subsequently tested and to assess which inhibitor is most capable of inhibiting collagenase.

***In silico* inhibition study methodology**

In order to develop or study potential new MMP-13 inhibitors, several *in silico* methodologies can be used, as it reduces the costs and time needed to develop a new inhibitor. In this case, an elaborate simulation consisted of a molecular study of Docking. A computational method that, using a mathematical structural search algorithm, tries to anticipate the best conformation of the link between two generally, a protein, in the case of this study the enzyme collagenase, and a ligand, in this study a library of potential collagenase inhibitors. In addition to the three-dimensional (3D) conformation of predicted binding of the potential inhibitor to the protein, which is usually called POSE; this algorithm also calculates, through various molecular parameters, a SCORE of the bond, which is a quantitative value of the prediction of the inhibitory potential of the inhibitory potential [8]. To carry out this type of Docking studies, it is important to choose, among the structures available in the PDB database, the most relevant collagenase structures. The more complete and correct for a 3D structure of the probability in question, in principle the better they will be as related by the Docking software.

Methodology

***In silico 37* compound library preparation**

A library of 37 compounds of the flavonoid class was created manually and individually in two-dimensional (2D) format using Marvin Sketch software and converted to a three-dimensional (3D) format in the same software. The 3D structure was optimized using a specific script from the VEGA ZZ software [2] and saved in pdb format. Using AutoDockTools (ADT) those pdb files were converted into pdbqt format, essential format to run a Doking study with AutoDock Vina (VINA) software.

Collection and preparation of collagenase 3D structures

Through the information available in the Protein Data Bank (PDB) database, all available 3D collagenase structures (MMP-13) were collected. The collected structures were then evaluated considering various parameters. Thus, structure 5B5O was selected and then prepared following a protocol for the use of VINA.

VINA software validation

A VINA control was done by using the rigid 5B5O rigid structure against the various ligands (designed and prepared in the same way of the 37 library compounds) of the remaining structures obtained in PDB database, that had, necessarily a connection affinity value associated, allowing to obtain the accuracy of VINA software, by correlating the experimental value with the obtained value by molecular Docking.

Therefore, some molecular dockings inducing flexibility with ADT in some key amino acids (glutamine 223, Leucine 184, Leucine 185, Threonine 245 and Leucine 218).

To perfume, Autodock VINA needs a tridimensional space to operate, called grid box. In this study the parameters used were the following: size of 40 Angströms (Å) for the three dimensions X, Y and Z, to cover the active site of the complete protein, and X, Y, Z coordinates of the center of the grid box with the values: 1.6; -10.7; 20.1.

Finally, an exhaustiveness value of 32 was defined, a value that influences the detail with which the VINA mathematical algorithm seeks the best connection conformation. These results are, in a final step, extracted and organized from the lowest to the highest predicted free energy value (expΔG), the lower this value, the greater the prediction of the compound's inhibition potential. Through these values it is possible to determine the values of the predicted inhibition constants (pki).

Results and Discussion

Since the 5B5O structure (Fig. 2) was the one with the lowest resolution value, 1,2 Å (the lower the resolution the better quality and detail of the structure) it was the structure choose for this molecular Docking study, having an associated ligand, with the WMM code (N-phenyl-4 - [(4H-1,2,4-triazol-3-sulfanyl) methyl] -1,3-thiazol-2-amine),

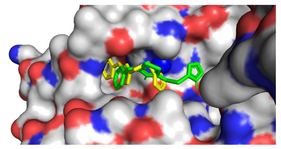

Figure 2 - Interaction of the co-crystallized WMM ligand (yellow) and experimentally predicted (green) at the active collagenase site. Figures customized and prepared using Pymol software (The PyMOL Molecular Graphics System, version 2.4 Schrödinger, LLC

Docking study using selective flexibility of collagenase

Before carrying out the Docking studies with compounds from the 37 flavonoids library, we were able to carry out a Docking study, in which we tried to consider the flexibility of some key amino acids in the collagenase catalytic zone. In this case, we flexed only a few amino acid residues, which were identified as being part of the active collagenase center: a Glutamate (Glu223), a Threonine (Thr245) and three Leucines (Leu184, Leu 185 and Leu218). All co-crystallized ligands were extracted from the respective experimental structures and prepared for Docking assays, a total of 38 ligands, with known experimental Ki (or equivalent) values were used. The pKi values predicted by VINA were correlated with the experimental values obtained for each inhibitor. The correlation coefficient (R^2) between the values predicted by VINA and the experimental values was calculated and are shown in Table 1.

R^2	All ligands	>1 nM	>10 nM	>100 nM
N° of Ligands	38	27	17	10
5B5O Rigid	0,0504	0,0459	0,2407	0,6258
Glu223	0,0264	0,0019	0,0152	0,4085
Leu184	0,0009	0,004	0,0328	0,3949
Leu185	0,0021	0,0013	0,1607	0,2933
Thr245	0,0016	0,0034	0,1625	0,2998
Leu218	0,0002	0,0076	0,1759	0,4337

Table 1 - Results of molecular Docking. In evidence the correlation coefficients (R^2) of the molecular Dockings between the rigid 5B5O structure and the same selectively flexible structure, and the co-crystallized ligands extracted from the respective experimental structures. For the total number of co-crystallized ligands, the highest value obtained was 0.0504 with the rigid structure. The compounds with inhibition Ki values less than 1 nM (27 compounds), less than 10 nM (17 compounds) and less than 100 nM (10 compounds) were removed from the correlation. The best correlation obtained was 0.6258 for the rigid structure when only compounds with Ki> 100 were considered.

When we consider all compounds, we find that the values of R^2 obtained are very low, either for the Docking with the rigid structure, or for the Docking studies with selective flexibility. However, the value of R^2 for the rigid Docking is higher (0.0504), when compared with the studies of Docking with selective flexibility. In general, we want the value of R^2 to be as close as possible to the value of 1. However, when analyzing the results, we observed that some of the co-crystallized inhibitors had a very high inhibitory capacity (with very low experimental Ki values), which suggested that VINA might not be able to predict Ki values for more potent compounds. Thus, we removed compounds with inhibition Ki values less than 1 nM from the correlation (only compounds with values >1 nM in a total of 27 are considered), less than 10 nM (only compounds with values >10 nM in a total are considered 17) and less than 100 nM (only compounds with values >100 nM in a total of 10 are considered). We recalculated the R^2 values for each situation and found that the best R^2 value obtained was 0.6258, in the situation where only the 10 compounds with Ki values greater than 100 nM were considered, using the rigid Docking (Fig. 3).

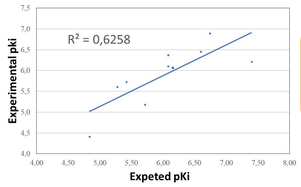

Figure 3 - Graph indicating the correlation (R^2) established between predicted pKi and experimental pKi of the rigid docking with the 10 compounds with an inhibition constant greater than 100 nM (Ki > 100 nM).

Taking this result into account, we used the rigid Docking to study the library of 37 flavonoids, since, with this approach, it was possible to obtain more reliable predictions.

Virtual screening

Since the flavonoid library was relatively large, we used an automation tool for the Docking processes, MOLA [9]. This software allows to automation of several processes associated with Docking. The use of MOLA allowed the automatic ordering of compounds with the highest inhibitory potential (lowest ΔG and Ki) for compounds with the lowest inhibitory potential (highest ΔG and Ki). Thus, we selected the four compounds with the greatest predicted inhibition potential (Table 2), Kaempferitrin (predΔG = -9,6 Kcal/mol), Nicotiflorin (predΔG, = -9,5 Kcal/mol), Rutin (predΔG = -9,4 Kcal/mol) and Broussoflavonol F (predΔG = -9,2 Kcal/mol), are two-dimensional structures are shown in figure 4. These compounds are those that we predict to have the best collagenase inhibitory capacity.

Composto	predΔG (Kcal/mol)
Kaempferitrin	-9,6
Nicotiflorin	-9,5
Rutin	-9,4
Broussoflavonol F	-9,2

Table 2 - 4 major flavonoids out of a total of 37 from the compound library with the greatest potential for collagenase inhibition (lowest predicted ΔG) after the virtual screening performed by VINA with automation using MOLA

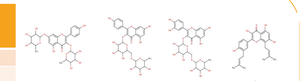

Figure 4 - Two-dimensional (2D) structures of the 4 flavonoids, from a total of 37 studied, with greater potential for collagenase inhibition.

When carrying out a Docking study, it is also possible to analyze the 3D conformation of the expected bond between the studied molecules and a protein structure, using visualization software such as Pymol. In this way it is possible to analyze what the occupation predicted by the compounds in the catalytic center, and which are the most important intermolecular bonds. In figure 5, we can see the connection conformation predicted, by the VINA software, for the flavonoid with the greatest inhibitory potential: Kaempferitrin.

It was possible to observe that, in the conformations predicted by VINA, the flavonoids bound to collagenase in an area immediately adjacent to the active center of the protein. This fact can may be explained by the fact that these flavonoids present large ring structures that are not able to occupy the pockets of the active center.

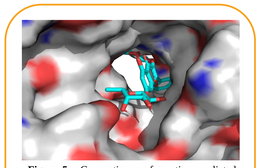

Figure 5 - Connection conformation predicted by the VINA of the flavonoid with the highest inhibitory potential: Kaempferitrin (-9.6 predΔG and predKi = 91.86 nM)

It is important to highlight that the first three flavonoids in the ranking have at least one rutinosis in their constitution. In fact, the compound in which a better prediction of binding is obtained, Kaempferitrine, has two residues of rutinosis. This location of rutinosis residues makes structural sense because, as rutinosis is quite polar due to the presence of hydroxyl groups in its structure, it probably forms hydrogen bonds with the solvent, that is, with water molecules.

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Film-Forming properties of fish collagen in the presence of plant extracts

Katarzyna Adamiak[1,2], Alina Sionkowska[1]

[1]Nicolaus Copernicus University in Torun, Faculty of Chemistry, Department of Biomaterials and Cosmetics Chemistry, Gagarin 7 street, 87-100 Torun, Poland

[2]WellU sp. z.o.o. Wielkopolska 280 street, 81-531 Gdynia, Poland

Introduction

Collagen constitutes the main structural protein of the living organisms [1,2]. Due to its biocompatibility [3-6], it is used in pharmacy, food, supplementation and cosmetic field [7-9]. Collagen in food industries comprises factor improving consistency, elasticity and stability of the products, whereas the main application is improving the alimentary value of food products [10]. On the other hand in cosmetics collagen is used as a humectant that meliorates skin's hydration, while its film-forming properties reduces the transepidermal water loss, smoothing, and brightening the skin [11]. Origin of collagen has an influential impact on its composition and safety of use.

The aim of this research was to determine the influence of *Melissa officinalis* herba addition on superficial properties of collagen films derived from fish collagen. Mechanical properties of the materials were also examined. Examination of the superficial and mechanical properties of collagen films is essential, because of the adhesion of to the human skin.

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Materials

Liophylysate of Silver Carp skin collagen was diluted in acetic acid.
Mellisa officinalis extract was diluted in water, then proper volume of the solution was transferred to the collagen solution.
From collagen solution individually and mixed collagen-melissa solution thin films were obtained.

Methodology

Superficial properties were examined using Atomic Force Microscopy and Scanning Electron Microscopy.
Mechanical properties were tested using Zwick&Roel machine.
Infrared spectroscopy allowed to assess the materials structure.
ATR mode was applied.

Results

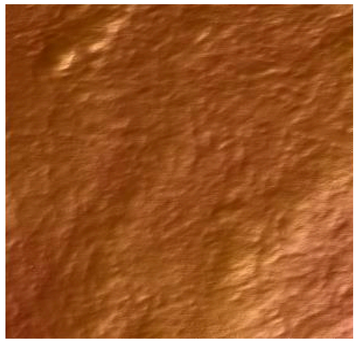

Figure 1. AFM visualization of collagen film

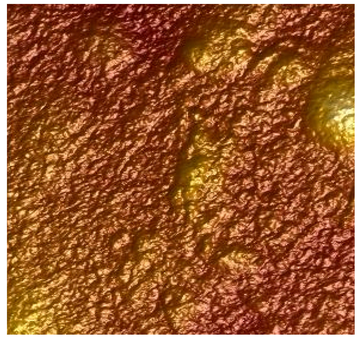

Figure 2. AFM visualization of Melissa-incorporated collagen film

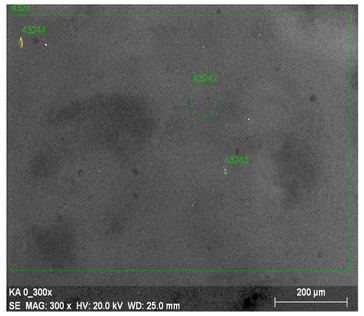

Figure 3. SEM visualization of collagen film

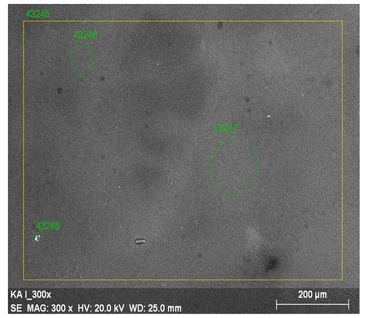

Figure 4. ASEM visualization of Melissa-incorporated collagen film

Conclusion

Atomic Force Microscopy and Scanning Electron Microscopy have shown that the addition of the *Mellisa officinalis* extract modifies superficial properties of collagen films.
Roughness of collagen films has been altered eminently as a result of addition Mellisa officinalis extract.
The influence of used plant extract on the mechanical properties of collagen film was also observed.
Superficial and mechanical properties of collagen films have influence on the adhesion to the human skin, which is significant in regard to biomaterial and cosmetic application.

NPA
Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

ELASTASE AND ITS POTENTIAL AS TARGET PROTEIN FOR BIOACTIVE COMPOUNDS

Luis Carlos Machado Sampaio[1], Filomena Adega[2], Rui M. V. Abreu[3]

[1]Escola de Ciências da Vida e Ambiente, Universidade de Trás-os -Montes e Alto Douro (UTAD), Quinta de Prados 5000-801, Vila Real, Portugal; [2]Departamento de Genética e Biotecnologia, Universidade de Trás-os -Montes e Alto Douro (UTAD), VilaReal, Portugal; [2]Centro de Investigação da Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia 5300-253, Bragança, Portugal.

Email: luis.carlos.sampaio98@gmail.com

Abstract

Elastase (MMP-12) is a metalloprotein of the matrix metalloproteinase (MMP) family and plays an essential role in various biological processes and, in deregulated situations, it is associated with pathological conditions such as cancer and pulmonary emphysema. It is also associated with aging and loss of skin elasticity, so it has been studied as a potential target for dermatological and cosmetic treatments. Hence the importance of compounds with an inhibitory capacity for therapeutic targets.

Due to the importance of developing inhibitors for elastase, it was discovered for example the potential of Ageladine A, from a marine sponge (Agelas nakamurai), which showed inhibitory capacity for MMP-12, as well as other members of the family of MMPs. In addition, Ageladine A derivatives were also synthesized to obtain compounds more effective in inhibiting elastase.

Knowing the generic structure of an elastase inhibitor, it is possible to investigate and potentially discover new inhibitors. For the identification of these inhibitors, we emphasize the importance of in silico tools. One of the most used are molecular docking tools, that have the advantage of being readily available, being fast and with the potential to replace, at least partially, experimental tests. In this work a molecular docking study was performed, using AutoDock Vina software, which made it possible to predict the inhibitory potential of a flavonoid library composed of 37 compounds of natural origin, against an elastase experimental three-dimensional structure. From the flavonoid compounds studied, Ombuin presented the lowest predicted binding energy (ΔG) with a value of -11Kcal/mol. This value indicates that this compound may be a potential elastase inhibitor, and it may be a promising compound in combating pathologies associated with elastase and in possible treatments in the cosmetic area.

Elastase and its potential as a target protein

The skin is composed of the epidermal, dermal and subcutaneous layers and its primary structural proteins are collagen and elastin. While, the former provides tensile strength, elastin is responsible for elasticity. (Wen et al. 2020).

Elastase, also known as macrophage metallo-elastase, is a metalloprotein of the matrix metalloproteinase (MMP) family, specifically it is member number twelve of the family (MMP-12) (EC 3.4.24.65. In general, elastase is involved in the degradation of extracellular matrix components, specifically elastin, in cases of physiological processes such as combating viral infections, in the inflammation process and in tissue remodeling (Du et al. 2020).

Elastase is involved in the gradual loss of skin elasticity, since this enzyme is responsible for the degradation of skin elastin, which causes loss of skin elasticity. Thus, it has also been studied as a potential cutaneous target for dermatological and cosmetic treatments (Imokawa and Ishida 2015). This metalloproteinase, having been associated with several pathologies, is considered an important potential therapeutic target in combating these pathologies. It has also been linked to the aging process. Thus, several studies have been carried out, to develop new inhibitors of this enzyme, to combat these associated pathologies and to be used in the cosmetic industry.

Known natural elastase inhibitors

The compound Ageladine A (figure 1A) extracted from a marine sponge, *Agelas nakamurai*, showed an inhibitory activity for MMP-12, although it also had presented inhibition ability for other members of the MMP family (Fujita et al. 2003).

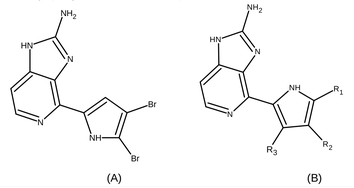

Figure 1 - (A) Structure of Ageladine A. **(B)** Base structure for the synthesis of new inhibitors. Structures prepared in ChemSketch software.

Due to the inhibitory activity of Ageladine A, (Ando and Terashima 2009), derivatives of it were also synthesized, in order to obtain compounds more effective in inhibiting elastase. The synthesis of new inhibitors went through the exchange of 3 radicals in the structure of Ageladine A (Figure 1B). Of the various synthesized inhibitors, 3 of them had a lower IC_{50}, compared to Ageladine A and, consequently, better inhibitory power.

In silico methods for the use of 3D elastase structures

For the development of elastase inhibitors, capable of becoming good inhibitors in the treatment of dermatological, pulmonary, cardiac and tumor diseases, different in silico techniques of structural bioinformatics are used. These methodologies are normally used before the experimental assays, and allow the prediction of inhibitory capacity, through bioinformatics tools, with the advantage of cost reduction and faster development of an inhibitor. (Froufe et al. 2013). For these studies it is necessary to choose the 3D structures of elastase, obtained in specialized databases such as PDB and the *in silico* preparation, either of the protein structure, or of the library of compounds to be studied as potential inhibitors. (Chaudhary and Mishra 2016). The preparation of elastase structure and the potential inhibitors is done in specialized software such as AutoDockTools. When it is necessary to design potential inhibitors, tools such as ACD / ChemSketch or Marvinsketch are used. (Schleinkofer et al. 2006); (Froufe et al. 2013). Having a 3D structure of elastase prepared and the library of compounds properly prepared, it is possible to carry out in silico studies of molecular modeling such as molecular docking, which is a computational tool with the objective of predicting the conformation of protein-ligand bonding. The docking methods are based on mathematical algorithms that in turn analyze various parameters to obtain the results in the form of a target protein inhibition score. So, we can see which compounds have the greatest inhibitory potential and proceed to their ranking. The compounds with the greatest potential can thus be studied experimentally to confirm or not the prediction of inhibition. (Chaudhary and Mishra 2016); (Schleinkofer et al. 2006).

Molecular docking of a small library of flavonoids against elastase

A molecular docking study was carried out to predict the potential inhibitor of a flavonoid library of natural origin against an elastase structure (Pradiba et al. 2018), using a molecular docking software AutoDock Vina (VINA). For molecular docking studies, a correct selection of the 3D elastase structures is necessary, and after choosing the structure, it is necessary to validate it, removing the co-crystallized ligand from the structure.

Then the elastase structure without the ligand was prepared, and the individual ligand was also prepared separately. The preparation of the protein and the ligand is carried out using the AutodockTools software. The files taken from AutoDockTools, referring to both protein and ligand, are presented in the PDBQT computational format, being ready to be used for *in silico* molecular docking studies. Then, the docking test was performed with the ligand, using the VINA software, which allows a comparison between the experimental 3D structure removed from the PDB and the structure provided by VINA. Then it is possible to perform a structural comparison, using the Pymol structure visualization software.

This comparison is shown in Figure 3 (A), in which we can observe an almost perfect overlap between the conformation predicted by VINA and the experimental conformation of the B9N inhibitor, obtained from the 6EKN structure. In this way, we validated VINA to be used for further docking studies, since it was able to predict the connection conformation between the B9N ligand and the elastase quite accurately. This type of study is usually carried out as a control to try to understand if the 3D structure of the protein can be used for docking assays with other compounds of interest. In this case, this control study was successful. After the protein structure was selected and validated, a library of 37 flavonoids, present in the study by (Pradiba et al. 2018), was prepared and designed.

To draw the two-dimensional (2D) representation of each compound, the MarvinSketch software was used and, for the three-dimensional (3D) representation was used the MarvinSketch and AutodockTools software (Figure 2).

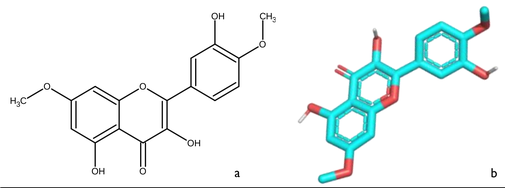

Figure 2 - Representation of Ombuin in: (a) 2D performed in MarvinSketch and (b) 3D visualized in Pymol.

Each compound was then stored in a PDBQT format, necessary for the following docking studies. All structures were carefully inspected at the end in the Pymol software in order to verify that the chemical structures of each flavonoid was correct. The docking study of each compound in the library was then carried out, using the same protocol previously used for compound B9N. When the molecular docking study is carried out for a large number of compounds, it is usually called "virtual screning".

Results

Of the 5 flavonoids presented in Table 1, all have the potential to be good elastase inhibitors, especially Ombuin, which proved to be a possible best inhibitor, with the lowest predicted ΔG value of -11.0 Kcal/mol. The 3D docked conformation predicted by VINA for Ombuin is shown in Figure 2B. So, we can see that VINA provides two types of results: (i) A docking conformation prediction, usually called POSE (Figure 2B). (ii) A quantitative value of the binding potential provided as a predicted ΔG value, which we usually designate as SCORE (Table 1).

Compound	Predicted ΔG (Kcal/mol)
Ombuin	-11.0
Tamarixetin	-10.8
Kaempferitrin	-10.8
Quercetin	-10.7
Rhamnetin	-10.7

Table 1 - 5 best results of the molecular docking assay with the protein (6EKN).
Legend: predicted ΔG - predicted Gibbs free energy

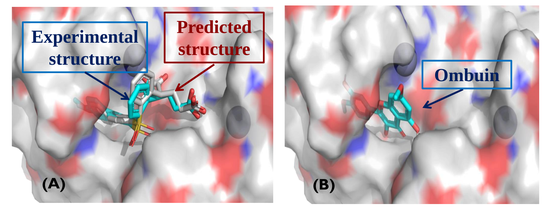

Figure3. (A) Overlapping of the structure of the co-crystallized B9N compound (experimental) and the predicted by molecular docking using VINA. **(B)** Predicted conformation by molecular docking of flavonoid Ombuin. Images prepared in PyMOL software.

Conclusion

For Ombuin, both POSE and SCORE provide indications that this compound is a potential elastase inhibitor and may be a promising compound in combating pathologies associated with elastase and in possible treatments in the field of cosmetics.

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Assessment Of Cosmeceutical Potential Of *Agaricus Brasiliensis* Mushroom: Antioxidant And Anti-Tyrosinase Activity

Maja Kozarski[1], Anita Klaus[1], Dragica Jakovljevic[2]

[1]Faculty of Agriculture, University of Belgrade,

[2]Institute of Chemistry, Technology and Metallurgy, University of Belgrade

Abstract

Mushrooms bioactive extracts are receiving increasing attention in the design of cosmeceutical formulations for topical application. In this study polysaccharide extract of *Agaricus brasiliensis*, a cultivated edible mushroom was screened for the free radical-blocking potential which could strengthen the skin's barrier function, and inhibition of tyrosinase which could provide a skin-lightening effect. The antioxidant activity of extract was evaluated by *in vitro* models including ABTS free radical scavenging activity, and inhibition of lipid peroxidation (LPx) in a linoleic model system. Carbohydrates were the most abundant components of the extract, and smaller quantities of proteins were detected. The β-glucan fraction represented 71% of carbohydrate content. β-glucans, despite their considerable molecular weight, are known to enter the *stratum corneum* and epidermis, penetrating deep into the dermis. Extract was found to be effective inhibitor of LPx, with an almost three-fold increased inhibition compared with ascorbic acid (EC_{50}=1.90 mg/ml), a common additive in cosmeceutical formulations used at mg levels. Likewise, extract showed moderate inhibition potential on tyrosinase compared to the anti-tyrosinase IC_{50} value of kojic acid (0.079 mg/ml), which is currently used in topical dermatological products. Cosmeceutical potential of investigate extract confirmed that *A. brasiliensis* may represent a promising source of natural cosmeceutical ingredients.

Introduction

Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the process of human skin aging. The skin is constantly exposed to atmospheric oxygen, ultraviolet (UV) irradiation, pollutants and xenobiotics. Oxidative damage caused by these exogenous sources can impair skin structure and function, leading to the phenotypic features of extrinsic aging. In addition, excessive consumption of alcohol, improper diet, physical inactivity and mechanical stress can contribute to oxidative damage of skin.

The endogenous defense systems against ROS are often insufficient to combat oxidative processes in mature skin. ROS that are not neutralized can target biomolecules and lead to cellular dysfunction or death and accelerated aging. It may also be an essential causative factor for hyperpigmentation or even carcinogenic processes in the skin.

Modern trends in the cosmetics industry prioritize ingredients or extracts from natural sources with nontoxic effects and the ability to delay the aging process. Mushrooms bioactive extracts are receiving increasing attention in the design of cosmeceutical formulations for topical application. The mushroom *Agaricus brasiliensis* is one of the most intensively studied medicinal mushrooms. It was distributed originally in Brazil and is presently cultivated in countries such as Korea, Japan, and China. *A. brasiliensis* is widely used today in Oriental countries both as an edible mushroom, considered a functional food, and as a natural therapy in the form of a medicinal extract mostly for prevention and treatment of cancer.

The objective of the present study was to investigate the potential therapeutic effect on mature skin of polysaccharide extracts from the fruiting bodies of a European source of *A. brasiliensis*. These effects are expected to influence oxidative stress via the potential antiradical activity of the extracts and inhibitory effect on tyrosinase which could provide a skin-lightening effect. .

Materials

Fruiting bodies of *A. brasiliensis* (strain M7700, Mycelia, Belgium) were analyzed. The fruiting bodies were harvested and air-dried in mature stage at 40 °C to constant mass, and ground into fine particles, which was stored in the dark prior to analysis.

Crude polysaccharide extracts were prepared by hot water extraction of 100 g of powdered sample. After centrifugation, the resulting pellets were washed with ethanol 70% (v/v), and dialyzed against Milli-Q water for 24 h at room temperature to remove residual small molecules as polyphenols, peptides and polysaccharides <8-10 kDa. After centrifugation, high molecular weight polysaccharides were ethanol precipitated and vacuum dried.

Methodology

Polysaccharide extract of *Agaricus brasiliensis* was screened for the free radical-blocking potential which could strengthen the skin's barrier function, and inhibition of tyrosinase which could provide a skin-lightening effect.

Cosmeceutical potential

The antioxidant activity of extract was evaluated by *in vitro* models including, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity, and inhibition of lipid peroxidation (LPx) in a linoleic model system. Results were expressed as EC_{50}(mg/mL) values, representing the effective concentrations of extract required to show 50% antioxidant activities. Extract was analyzed at the concentration range of 0.04-5 mg/mL. Ascorbic acid and EDTA were used as positive controls.

Tyrosinase activity was determined in a 96-well plate using an absorbance microplate reader (ELx808, BioTek Instruments, Inc., USA) controlled by Gen5TM Software to measure absorbance at 475 nm. An aliquot of the extract (40 µL) in 0.067 M of phosphate buffer (pH 6.8) containing 5% DMSO was incubated with 80 µL of phosphate buffer (pH 6.8) and 40 µL of tyrosinase (46 units/mL) at 23°C for 10 min. Next, 40 µL of 2.5 mM L-DOPA in 0.067 M of phosphate buffer (pH 6.8) was added. Extract was analyzed at the concentration range of 0.04-5 mg/mL, using kojic acid as a reference. The anti-tyrosinase activity of the extract was expressed as IC_{50} value, which was calculated using linear regression analyses as the concentration of extract required for 50% inhibition *in vitro*.

Chemical characterization

The total polysaccharide content of the extract was determined using the phenol-sulphuric acid method; the results are expressed as mg of glucose equivalents (GlcE) per 100 mg of dry weight (DW) of the extract.

The content of the total β-glucans was determined using the mushroom and yeast β-glucan assay procedure (Megazyme Int.); the glucan content is expressed as mg of GlcE per 100 mg of DW of extract.

The protein content was determined using the Bradford method; the total protein content is expressed as g of bovine serum albumin equivalents (BSAE) per 100 g of DW of extract.

Results

Carbohydrates were the most abundant components of the extract, and smaller quantities of **proteins** were detected (**Table 1**). Deproteinization of the polysaccharide fraction was not achieved with thermal treatment and dialysis was also insufficient for the removal of all proteins. **The β-glucan** fraction represented 71% of carbohydrate content. β-glucans, despite their considerable molecular weight, are known to enter the *stratum corneum* and epidermis, penetrating deep into the dermis. Within the dermis, they can stimulate collagen synthesis through direct interaction with fibroblasts and through indirect cytokine-mediated interaction with macrophages. Collagen synthesis is one possible mechanism by which the elasticity of the skin is enhanced. Extract was found to be effective inhibitor of **LPx**, with an almost three-fold increased inhibition compared with ascorbic acid (EC_{50}=1.90 mg/ml), a common additive in cosmeceutical formulations used at mg levels. Likewise, extract showed moderate **inhibition potential on tyrosinase** (**Table 1**) compared to the anti-tyrosinase IC_{50} value of kojic acid (0.079 mg/ml), which is currently used in topical dermatological products.

Table 1: Chemical composition, antioxidant and anti-tyrosinase potential of *A.brasiliensis* polysaccharide extract

Properties	
Chemical composition	**mg/100mg ± SD**
Carbohydrates	64.7 ± 0.7
β-glucan	45.1 ± 0.5
Protein	7.3 ± 0.4
Cosmeceutical potential	
Antioxidant activity	**EC_{50} (mg extract/ml) ± SD**
ABTS	0.09 ± 0.001
LPx	0.65 ± 0.04
Enzyme inhibition	**IC_{50} (mg extract/ml) ± SD**
Anti-tyrosinase activity	1.23 ± 0.02

Conclusion

The polysaccharide extract of *A. brasiliensis* may represent promising alternative raw materials for use in cosmetic products. However, these findings require further verification in clinical trials to examine the stability, skin permeation, and efficacy of the final cosmeceutical product.

THE NATURAL COSMETICS

Recommendations

The results of the present study strongly support the existing scientific data on the use of ingredients from natural sources as anti-aging agents and their application in the cosmeceutical industry.

Acknowledgements

This work was supported by a contract for the realization and funding of research work in 2020, between the University of Belgrade - Faculty of Agriculture and the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number: 451-03-68 /2020-14 / 200116.

CONSUMERS BEHAVIOR AND PERCEPTIONS REGARDING NATURAL COSMETIC PRODUCTS

Fernanda Possamai[1], Sarra Jelidi[1], Briolanja dos Santos[1], Olívia R. Pereira[2], Paula Cabo[2], Maria João Sousa[2]*

1 School of Agriculture, Polytechnic Institute of Bragança,Campus of Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal; sarrajelidi7@gmail.com;

2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; oliviapereira@ipb.pt; paulacabo@ipb.pt; joaos@ipb.pt;

*Correspondence: joaos@ipb.pt; Tel.: +351-273-303-200

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Abstract

In the last decades, the increasing concerns related with sustainability and the emergence of ethical concerns related to the production and consumption of cosmetics, as well as, the verification of the adverse effects of chemical additives present in cosmetics for human health and the environment, have driven the growth of green cosmetics. Natural substances, derived from plants, animals or other organisms are increasingly popular as ingredients in cosmetics for being considered by consumers as safety alternatives to synthetic cosmetics. This work aims to analyze the habits and perceptions of consumers regarding natural cosmetics products. A cross-sectional study was carried out based on a sample of 224 individuals between 18 and 74 years old. Respondents were mainly female (75%), young urban adults, employed, with higher education. Most of the respondents use cosmetics daily, and considers them important (48%) or essential (34%), particularly the hygiene and skin care products. The median amount of monthly spend on cosmetic products are of 20 €. Super and hypermarkets (34.5%), and pharmacies and parapharmacies (31%) are the most relevant places for buying cosmetic products although the internet (8%) and catalogues sales (7%) are also significant. The importance of internet is also present has source of information about the products (40%), seconded by beauty professionals (27%). The great majority of the respondents uses natural cosmetic products, although not often organic ones. Despite the respondents' considerable familiarity with natural cosmetic products, results display some misconceptions about these products is still present in consumers' minds.

Introduction

Since ancient times, Man has drawn on nature in search of ingredients that he can use to maintain his body hygiene and his good appearance, hence the evolution of cosmetics to the point where analogues to bioactive molecules was chemically synthesized. According to archeological studies, the use of cosmetics dates back to ancient mankind, since the early Stone Age (Joshi & Pawar, 2015). Over the years, natural products became a modern trend in the field of beauty since consumers have shown preference for natural products and more awareness of chemicals. Unlike traditional synthetic products, different plant parts and plant extracts are used in these products such as Aloe vera gel or coconut oil. Compared to chemically synthesized beauty products, natural cosmetics are estimated safe to use (Mitsui, 1997). Dermatologists tested them and proved their hypoallergenic criteria. Therefore, consumers of such products have less risk of getting skin rashes or experience skin itchiness. In the last decades, the increasing concerns related with sustainability and the emergence of ethical concerns related to the production and consumption of cosmetics, as well as, the verification of the adverse effects of chemical additives present in cosmetics for human health and the environment, have driven the growth of green cosmetics. Natural substances, derived from plants, animals or other organisms are increasingly popular as ingredients in cosmetics for being considered by consumers as safety alternatives to synthetic cosmetics. This work aims to analyze the habits and perceptions of consumers regarding natural cosmetics products

This work aims to analyse the habits and perceptions of consumers regarding natural cosmetics products.

Methodology

The study was carried out through a self-administered questionnaire, previously structured according to the objectives of this study. The study were made with the informed consent of the respondents, ensuring all the constitutional values and rights of the individuals. Respondents were previously informed about the voluntary nature of their participation, about the research objectives. The information collected was treated in order to guarantee confidentiality and anonymity and the data exclusively used for the scope of this investigation. The questionnaire consists of four sections, the first section contains sociodemographic questions to obtain general information and lifestyles of the respondent, in the second section it is intended to evaluate skin care, the third section is facial self-assessment of the respondent, while the fourth - determine the level of knowledge of natural cosmetic products. Data collection was carried out online, during the month of November, 2020.

Materials

The cross-sectional study was carried out based on a sample of 224 individuals between 18 and 74 years old. Respondents were mainly female (75%), young urban adults, employed, with higher education (**Table 1**)

Table 1: Sample Description

Variable	Category		Frequencies N	Frequencies %
Age	<25 years old		36	16,1%
	25-35 years old		111	49,6%
	35-45 years old		45	20,1%
	45-55 years old		17	7,6%
	>=55 years old		14	6,3%
	Non response		1	0,4%
Gender	Male		55	24,6%
	Female		168	75,0%
	Non response		1	0,4%
Schooling	3rd Cycle of Basic Education or lower		8	3,6%
	Secondary School		36	16,1%
	Post-secondary technological course or Bachelor		2	0,9%
	Degree or higher		176	78,6%
	Non response		2	0,9%
Marital Status	Single		148	66,1%
	Married or cohabiting		59	26,3%
	Separated / divorced		8	3,6%
	Widow		6	2,7%
	Non response		3	1,3%
Professional Status	Unemployed		7	3,1%
	Student		64	28,6%
	Employeed		120	53,6%
	Self-employed		32	14,3%
	Retired / Pensioner		1	0,4%
Household Size	1 or 2 persons		136	60,7%
	3 or 4 persons		77	34,4%
	5 or more persons		9	4,0%
	Non response		2	0,9%
Montly Household Income	Any less de 500 €;		10	3,7%
	500 to 1000 €;		33	12,4%
	1000 to 3000 €;		119	44,6%
	3000€ or higher		22	8,2%
	Non response		40	15,0%
Place of residence	Portugal	Urban area	159	71,0%
		Rural area	29	12,9%
	Other country	Urban area	23	10,3%
		Rural area	3	1,3%
	Non response		10	4,5%

Results

Most of the respondents use cosmetics daily (68%) (**Figure 1**).The median amount of monthly spend on cosmetic products is 20 €, distributed as shown in **Figure 2**. For most of the respondents cosmetic products are important (48%) or essential (34%) (**Figure 3**), particularly hygiene and skincare products (**Figure 4**).

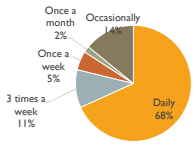

Figure 1: Frequency of use of cosmetic products (N=181)

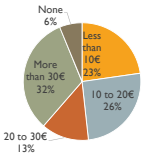

Figure 2:Average monthly expenses on cosmetic products (N=111)

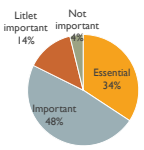

Figure 3: Importance of cosmetic products (N=181)

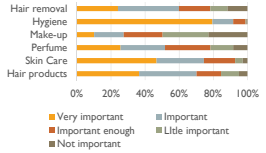

Figure 4: Importance of some specific cosmetic products (N=180)

Super and hypermarkets (34.5%), and pharmacies and para pharmacies (31%) are the most relevant places for buying cosmetic products although the internet (8%) and catalogs sales (7%) are also significant. The importance of the internet is also present as a source of information about the products (40%), seconded by beauty professionals (27%). The great majority of the respondents use natural cosmetic products, although not often organic ones **(Figure 5).** Despite the respondents' considerable familiarity with natural cosmetic products (only 4% of the respondents never use natural or organic cosmetic products), results display some misconceptions about these products are still present in consumers' minds **(Figure 6).**

Figure 4: Habits of using natural and/or organic cosmetic products (N=177)

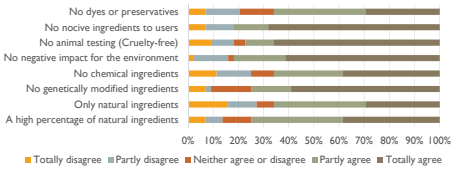

Figure 5: Perceptions of about natural cosmetic products attributes (N=44)

The main motivation for buy/use natural cosmetic products is health and environmental concerns (**Figure 7**). It should be also noted the role of social media (bloggers and YouTubers in influencing respondents' decisions.

Figure 7: Main reasons for buy/use natural cosmetic products

Conclusion

Consumers' interest in health and environmental issues is increasing, providing a huge opportunity for the natural and organic personal care industry to create a strategy that could motivate many consumers to purchase organic or natural personal care products. This study has made a contribution to existing knowledge about the industry of natural products by indicating the habits and perceptions of consumers regarding natural cosmetics products, that impact consumers' purchase intentions towards natural cosmetic products.

The study exposed the importance of cosmetic products for the consumers, has most of the respondents use cosmetics daily, and considers them important or essential, investing a significant part of the monthly budget to these expenses. Despite de traditional market places still prevailing (Super and hypermarkets, and pharmacies), the results highlighted the increasing importance of e-commerce and social media as influencers in consumers' decisions. The study also demonstrates the popularity of natural cosmetics, as most of the respondents use natural cosmetic products, although not often organic ones. However, despite the respondents' considerable familiarity with natural cosmetic products, results display some misconceptions about these products still present in consumers' minds, particularly, regarding the composition of natural cosmetic products and their effect on the environment.

Recommendations

Regardless of the positive growth trend in the world, the natural cosmetics market, particularly in Portugal, is still an under-researched area. Future investigation is still required to determine which variables influence consumer purchase intentions towards natural cosmetics.

Additionally, the misconceptions still prevailing on consumers about natural cosmetic products are an important issue that must be addressed by industry stakeholders has health and environment are the main reasons for consumers' choice of natural products.

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Acknowledgements

The authors would like to thank the Foundation for Science and Technology (FCT, Portugal) and the ERDF under the PT2020 program for financial support to CIMO (UID / AGR / 00690/2019).

NPA
Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

PRODUCTION AND *IN VIVO* SKIN HYDRATION EVALUATION OF A COSMECEUTICAL CONTAINING *OPUNTIA FICUS-INDICA* (L.) MILL CLADODES HYDROALCOHOLIC EXTRACT

Marta Cunha[1], Bárbara Ferreira[1,2], Luís Giraldo-Silva[1,2], Alberto C.P. Dias[1,2,3]

[1]Department of Biology, Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal.
[2]Department of Biology, AgroBioPlant Group, Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB-UM), University of Minho, Braga, Portugal.
[3]Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal.

NPA
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Online Edition
4-5 Feb 2021

Introduction

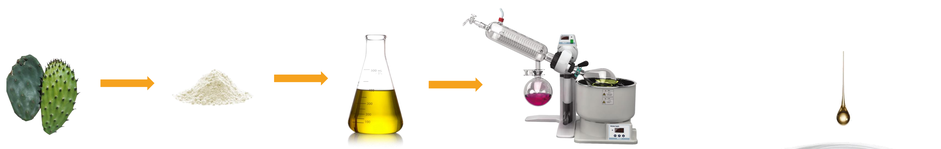
The term cosmeceuticals involve cosmetics with bioactive ingredients that provide pharmaceutical benefits to the skin (1). The moisturizers play an important role in repairing the skin barrier function. Furthermore, the preservation of stratum corneum integrity is essential for hydration maintenance. The water is important for hydrolytic enzymatic action needed for skin normal desquamation. Dry skin leads to adhesion and accumulation of corneocytes on the skin surface, arising a visible appearance of dryness, roughness, scaling, and flaking (2).

Plants extracts have been very present as ingredients in cosmeceuticals formulations. Several studies demonstrated that several plants extract are involved in the reparation of the skin barrier and the keratinocyte differentiation after topical application *in vivo* (3).

Opuntia ficus-indica cladodes are a valuable source of bioactivities related to skin health promotion, namely UV protection, wound healing and moisturizing effect (4, 5, 6). For this study, a cladodes hydrochloric extract (CHE) was made to be incorporated in a cosmeceutical formulation with the aim of evaluating the hydration potential and ensuring its safety.

Material and Methods

Cladodes from *Opuntia ficus-indica* were lyophilized, powdered and submitted to an ethanolic (80 %) extraction. The resulting extraction was concentrated in Rotavapor (Buchi RE-121) at a temperature of 40±1°C and lyophilized for 72 hours. Thereafter, a cosmeceutical formulation was developed for the incorporation of CHE.



CHE was used at 0.15 % as an ingredient in a cosmeceutical formulation.

Courage-Khazaka electronic GmbH, Köln, Germany.

Two different creams were manufactured for the *in vivo* skin evaluation, the cladode cream with CHE and the base cream containing distilled water instead of CHE to use as a control. Four healthy volunteers* with ages between 25 to 36 years were recruited for this study. In the right inner forearm and upper arm were delineated three areas: base cream, cladode cream, and the untreated (without any cream application), as it showed in the bellow scheme. For the aim of this study, the parameters hydration and erythema were evaluated using two non-invasive probes the Corneometer® and Mexameter®, respectively, from Courage-Khazaka electronic GmbH, Köln, Germany apparatus.

In vivo skin evaluation procedure

Delineation of the areas:
U- Untreated
BC- Base cream
CC- Cladode cream

At **0h** measures were taken without any previous cream application.

Cream application in the areas **BC** and **CC.**

Measures were taken **3h** after cream application and **6h** with previous cream reapplication at **3h .**

*The method, efficacy and possible side-effects of this study were explained to the volunteers and we obtained their consent to proceed.

Acknowledgements

The author acknowledges the financial support provide by the European Social Fund and the North Regional Operational Program 2014/2020: Norte 08-5369-FSE-000054 (ON 0018). This work was supported by Multiannual Financing ofUnits of I&D UIDB/04050/2020.

Results and Discussion

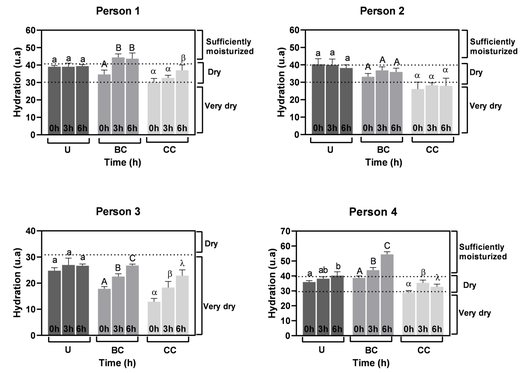
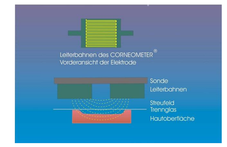


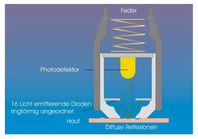
Figure 1. Hydration level (u.a) in the 3 different areas (untreated (U), base cream (BC) and cladode cream (CC)) at 0, 3 and 6 hours in each volunteer (Person 1, Person 2, Person 3 and Person 4). Each bar represent mean ± SD. There are significant differences from P≤0.05, statistically analyses by Two-Away Anova.

The Corneometer® principle is based on the capacitance method, the probe transmits an electromagnetic field that penetrates the skin surface (stratum corneum layer) and the water present in stratum corneum reflects the electromagnetic field that is detected by the probe. (Courage-Khazaka electronic GmbH, Köln, Germany manual).



The results from the measures of Corneometer ® are presented in figure 1. The persons were analysed separately due to the intrinsic differences in each person's skin. Figure 1 shows an increase of hydration values in the base cream (BC) application area for the persons 1, 3 and 4. The area treated with cladodes cream for the person 2, did not showed significant differences along the treatment. Besides, for person 1 and 4, only a slight increment of hydration values was observed. Although, stands out for the person 3 with very dry skin condition, a hydration improvement of 42 % after 3 hours of cream application and 25 % at 6 hours after reapplication.

Overall, both creams showed hydration effect on the skin, despite that in some cases the skin conditions still dry/very dry, suggesting that may be necessary a prolongation of the treatment to obtain a skin sufficiently moisturized.



The Mexameter® measure the reflectance based on absorption/ reflection from the skin, at wavelengths green and red measure the hemoglobin (erythema), wavelengths red and near-infrared measure the melanin. (Courage-Khazaka electronic GmbH, Köln, Germany manual).

To determine the possibility of skin allergy from both creams, only the erythema values were analysed.

The results from the measures of Mexameter ® are presented in figure 2. The persons were analysed separately due to the intrinsic differences in each person's skin. The erythema values could differ among healthy persons. According to the manual Courage-Khazaka electronic GmbH, Köln, Germany, the erythema values between 0-170 means no erythema and 170-330 means minimal erythema. In our study, there are healthy skin persons with values above 170 and no visible erythema was observed showing to be normal values of their healthy skin.

The erythema values did not show a linear behavior during the treatment, the untreated area revealed ambiguous variations for all the persons, except for person 4 that showed no significant differences to all areas. Temperature, physical and mental activity could be related to the non-linear variations since these factors exert a considerable influence on skin colour due to the blood flow alterations (7). On the other hand, we observed that the erythema values of areas base cream (BC) and cladodes cream (CC) did not increase compared to the 0 hours. Overall, the results indicate that both base cream and cladode cream does not cause skin allergic reactions.

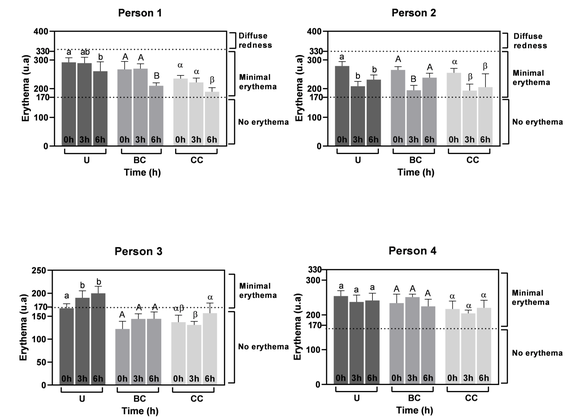


Figure 2. Erythema level (u.a) in the 3 different areas (untreated (U), base cream (BC) and cladode cream (CC)) at 0, 3 and volunteer (Person 1, Person 2, Person 3 and Person 4). Each bar represent mean ± SD. There 6 hours in each are significant differences from P≤0.05, statistically analyses by Two-Away Anova.

Conclusion

Although the current study is based on a small sample of volunteers, the results indicate that the cosmeceutical containing CHE had hydration potential, and no allergenic reactions were observed. Further studies are needed with more volunteers and could be directed to the treatment of critical conditions of dry skin like xerosis.

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APPLICATION OF CHITOSAN AND CHITOSAN DERIVATIVES IN COSMETICS

Marta Szulc, Katarzyna Lewandowska
Department of Biomaterials and Cosmetic Chemistry,
Nicolaus Copernicus University, St. Gagarina 7 Torun, Poland

Abstract

The growing consumer awareness of environmental protection and ecology resulted in an increased interest in natural cosmetics. This is a stimulus for scientists who are looking for natural polymers with a wide range of properties. Chitosan and its derivatives are biopolymers made from post-production waste such as crab or shrimp shells. It is also possible to obtain chitosan from mushrooms. Chitosan is non-toxic, biocompatible, biodegradable, can bind fat, and has antibacterial, antifungal and wound healing properties. In cosmetology, it can be used as a hair smoothing, moisturizing or antibacterial substance [1].
Carboxymethyl chitosan is a derivative of chitosan in which the carboxymethyl group is attached to the amino group or to the hydroxyl group. As a result, the following can be obtained: N, N-carboxymethyl chitosan, N, O-carboxymethyl chitosan, O-carboxymethyl chitosan, N-carboxymethyl chitosan and mixtures thereof. Carboxymethyl chitosan is biocompatible and biodegradable. It has antibacterial and antioxidant properties and can chelate metals. In cosmetic products, it can be used as a rheology modifier, an antibacterial substance or as an antioxidant substance [2,3].
In the conducted preliminary studies, the conditions for carboxymethyl chitosan synthesis were selected, which allow to obtain chitosan derivatives attractive from the point of view of cosmetic products. Their characteristics such as average molecular weight, degree of deacetylation (DA), degree of substitution (DS), were compared with characteristics of chitosan.

Introduction

Carboxymethyl chitosan (CMCS) is a chitosan derivative in which the carboxymethyl group is attached to the amino group or to the hydroxyl group.
Chitosan derivatives can be achieved such as:
N,O-carboxymethyl chitosan,
O-carboxymethyl chitosan,
N-carboxymethyl chitosan,
N,N-carboxymethyl chitosan.

Carboxymethyl chitosan is:
- highly viscous,
- biocompatible,
- biodegradable,
- non-toxic,
- water-soluble.

It has antioxidant and antibacterial properties.

In cosmetic products, it can be used as:
- a rheology modifier,
- an antibacterial substance
- an antioxidant substance.

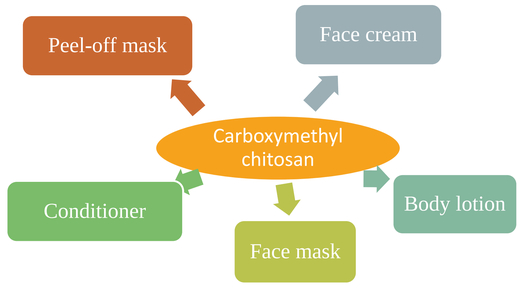

Fig. 1. Application of carboxymethyl chitosan

Methodology

Synthesis 1 and 1.2
2.0g chitosan (1.2 fragmentation chitosan) was added into 7.6g 40% NaOH aqueous solution. 20 mL isopropyl alcohol with 3.4g chloroacetic acid were added into the flask. Afterwards mixture was in under reflux at 65°C for 4 h. After cooling, impurities were removed by filtration. The product was precipitated by the addition of 80% ethanol. The product was then dried under vacuum.

Synthesis 2
2.7g of sodium hydroxide was dissolved in 4 mL of H2O and 16 mL of isopropanol and mixed with 2.0g of chitosan. The solution was left at room temperature for 1 h. Next, 3.0g of chloroacetic acid in 4 mL of isopropanol were dropwise added to the mixture for 30 min. Later mixture was in under reflux at 55°C for 4 h. After cooling, impurities were removed by filtration. The product was precipitated by the addition of 80% ethanol. The product was then dried under vacuum.

A Ubbelohde viscometer was used to determine viscosity average molecular weight. For the samples of CMCS sodium salts, 0.1M NaCl was used as a solvent, the test was carried out at 30°C.
For the chitosan sample, a mixture of 0.1M acetic acid and 0.2M NaCl was used, the test was performed at 25°C.

The degree of deacetylation and the degree of substitution were determined by performing a potentiometric titration. CMCS and chitosan samples were dissolved in 0.1M HCl, 0.1M NaCl was used for titration.

A Vertex 70V FT-IR spectrometer with a Hyperion 1000/2000 microscope from Bruker Optik was used for the spectroscopic analysis of the polymer films.

Results

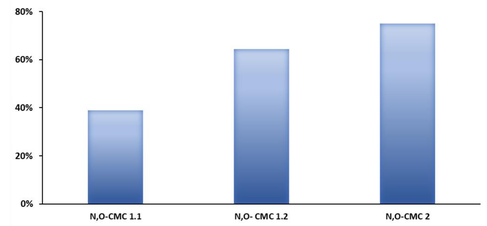

Fig. 2. Comparison of the efficiency of syntheses.

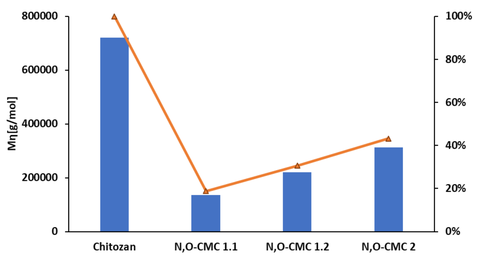

Fig. 4. Comparison of the viscosity average molecular weights of the obtained CMCS (blue). Comparison of the reduction in molecular weight of the obtained CMCS (orange).

Table 1
Values of the deacetylation degree (DD) and degree of substitution (DS).

	DD	DS
Chitozan	75%	-
N,O-CMCS 1	29,12%	87%
N,O-CMCS 1.2	38,99%	61%
N,O-CMCS 2	33,11%	45%

Conclusion

- The fragmentation of chitosan cause increased the reaction efficiency and the molecular weight of the polymer.
- The fragmentation of chitosan negatively influenced the degree of substitution.
- Adding an hourly to swelling, reducing the reaction temperature by 10 °C increased the reaction yield, the molecular weight of N, O-CMC but decreased the degree of substitution.

Recommendations

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Acknowledgements

The project was financed by the Young Scientists Grant 492/2020.

Stability of nanodispersions containing *Myrciaria cauliflora* Mart. extract for skin lightening products application

Nathalia Olivia de Sousa Garcia[1]; Edemilson Cardoso da Conceição

[1]nathalia.sousagarcia@gmail.com

Universidade Federal de Goiás, Faculdade de Farmácia

Introduction

Myrciaria cauliflora Mart. is popularly known as jabuticabeira [1]. Among the secondary metabolites present in *Myrciaria cauliflora*, ellagic acid can be found mainly in the peels of the fruit [2].

Figure 1: *Myrciaria cauliflora* M. Source: Own author.

Ellagic acid has antioxidant activity and capacity to complex with copper which gives the ability to inhibit the tyrosinase enzyme being able to affect production of melanin [3].

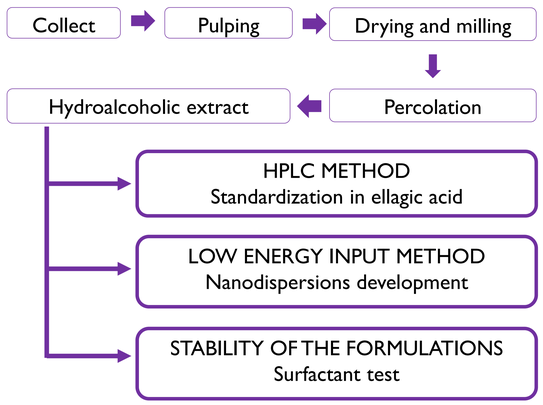
The application of nanotechnology associated with plant extracts may increase ability to lightening skin blemishes due to the capability to promote improvements in delivery system aspects by achieving greater speed of action, increasing the synergy with the skin and permeation of active compounds resulting on greater hydration power and differentiated effectiveness [4].

Aim

Develop nanodispersions from the extract of *Myrciaria cauliflora* fruit peels and evaluate their stability

Methodology

Figura 2: Flowchart of liquid extract production, standardization of ellagic acid extract, development and stability of nanodispersions.



Results

Table 1: Particle size, polydispersity index and zeta potential of nanodispersions prepared with Polysorbate 85 (A) and Polysorbate 80 / Sorbitan oleate 80 (B) on the day of preparation and after 7 days.

	Day one		
	Particle size (nm)	PDI	Zeta Potential (mV)
A	183,8 ± 2,12	0,228 ± 0,006	- 10,0 ± 0,26
B	226,8 ± 5,92	0,410 ± 0,011	- 12,4 ± 0,06
	Day 7		
	Particle size (nm)	PDI	Zeta Potential (mV)
A	186,4 ± 1,02	0,238 ± 0,006	-10,7 ± 0,25
B	231,9 ± 5,35	0,378 ± 0,042	-12,8 ± 0,28

Polysorbate 85 proved to be more efficient in stabilizing the formulation.

Nanodispersion prepared with polysorbate 85 presented smaller particle size and greater homogeneity between them along the study.

Figure 3: Hydroalcoholic extract of Myrciaria cauliflora and production of nanodispertions by low energy method

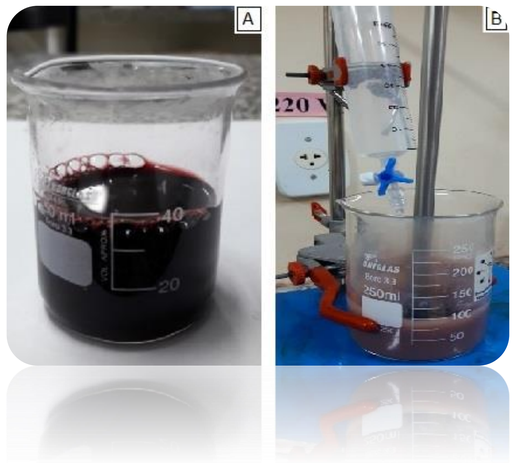
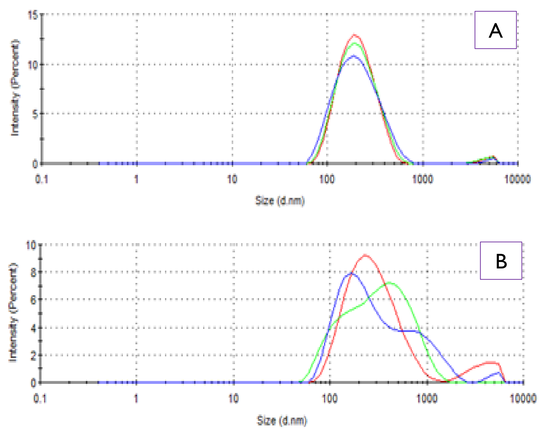


Figure 4: Particle size distribution of nanodispersions prepared with Polysorbate 85 (A) and Polysorbate 80 / Sorbitan oleate 80 (B) after 7 days.



Conclusion

The application of nanotechnology on extracts can increase the stability of the secondary metabolites of interest and their efficiency when applied on the skin therefore the stability of the formulation it is important to achieve an increased lightening action on skin blemishes therefore the selection of surfactants composition is an important step in the development of the skin lightening active formulation.

Recommendations

The development of nanodispersions for cosmetic application it is an important part of a new active production; The following study applying the nanodispersions formulations developed must be done to ensure its effectiveness in lightening skin blemishes;

Preliminary stydies have been already conducted in order to evidence its action on melanogenesis.

Acknowledgements

USERS SELF-ASSESSMENT OF A DAILY MOISTURIZING OF THE SKIN, WITH NATURAL INGREDIENTS

Sarra Jelidi[1], Olívia R. Pereira[2], Paula Cabo[2]*, Maria João Sousa[2]

[1]School of Agriculture, Polytechnic Institute of Bragança,Campus of Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal; sarrajelidi7@gmail.com;

[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; oliviapereira@ipb.pt; paulacabo@ipb.pt; joaos@ipb.pt;

*Correspondence: paulacabo@ipb.pt; Tel.: +351-273-303-200

NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Abstract

Nowadays, sustainability awareness is gaining relevance in the cosmetic sector. The increasing market trend in favour of green cosmetic products, which use ingredients from natural inputs, portrays the ecological and social weight of consumer choices when using products that contribute to nature in a sustainable way. This study aims to analyze the user self-assessment effectiveness of a cream formulation for daily moisturizing of the skin, with natural ingredients, including bee products, local plant material of the region of Trás-Os-Montes, and vegetable oils. Twenty-two individuals aged between 24 and 74 years were included in the product's effectiveness study. The pre-use self-assessment shows that most of volunteers presented mixed skin type (at least three in five individuals). For dry skin individuals (one in five individuals), skin jerking and the rapid absorption of the products are the main issues. The assessment of product efficacy was measured based on a post-use questionnaire. The results obtained for non-placebo users demonstrate that the product exhibits positive performance for all the attributes under assessment. For products efficacy the highlight is for hydration and skin smoothness. For products attributes, ease application scores the maximum of 3.3 points. Different, wrinkles reduction and fragrance present less favourable results. When asked about the willingness to buy the product only one in four users respond negatively, mainly because of the strong fragrance of the preservative agent product. Users willing to buy/recommending the product to a friend mention the satisfactory results obtained, hydration performance and natural product as the determinant for that decision. The users willing to buy the product consider to do it at an average price of 10€ for a 50ml package.

Introduction

Nowadays, sustainability awareness is gaining relevance in the cosmetic sector. The increasing market trend in favour of green cosmetic products, which use ingredients from natural inputs, portrays the ecological and social weight of consumer choices when using products that contribute to nature in a sustainable way. The natural cosmetics market can be an important opportunity for the valorization of the endogenous natural resources and creation of jobs in low-density, mountain regions, such as Trás-Os-Montes.

Cosmetics Europe (2019) refers that European consumers spend, on average, 135€ per year purchasing cosmetic products. The increase in the life span and mindset changing open a new and profitable market segment for the cosmetic industry. As this trade association highlights, the literature review indicates that average annual spend on cosmetics increases by age, such that older consumers spend considerably more than their younger counterparts, e.g., European women over the age of 60 spend three times as much on skincare as women under 25 (Credit Suisse, 2013). In 2018, the European natural cosmetics market share was (valued at 3.6 billion €) of less than 5% of the European cosmetics market. However, this market is growing faster than the overall cosmetics market. Skincare is the most important product category, in which a wide range of products use natural ingredients. The leading country markets for natural personal care products are Germany, France, Italy, and the UK. These countries comprise almost 80% of the European natural and organic personal care products market (CBI, 2020).

This study aims to analyze the user self-assessment effectiveness of a cream formulation for daily moisturizing of the skin, with natural ingredients, including bee products, local plant material of the region of Trás-Os-Montes, and vegetable oils.

Methodology

The effectiveness of the product was verified through an online questionnaire addressed to the study volunteers after 15 days of using the product.

The recruitment of the volunteers for the user self-assessment test was based on the results of a questionnaire addressed the users of cosmetic products, administered by electronic means, in order to identify the profile and habits of using facial care products. The study was carried out through a self-administered questionnaire, previously structured according to the objectives of this study. The study were made with the informed consent of the respondents, ensuring all the constitutional values and rights of the individuals. Respondents were previously informed about the voluntary nature of their participation, about the research objectives. The information collected was treated in order to guarantee confidentiality and anonymity and the data exclusively used for the scope of this investigation. Data collection was carried out online, during the month of November, 2020.

Materials

Twenty-three individuals aged from 24 to 74 years were selected for the product's effectiveness study. **Table 1**, and **Figures 1 to 3** present a brief description of the volunteers' socioeconomic and physical condition, lifestyles, and skincare profile. For skin type, most of the volunteers present mixed skin type (at least 3 in 5 individuals): for the 20% of dry skin volunteers, skin jerking and the rapid absorption of the products are the main issues. 30% of volunteers referred to have visible wrinkles (30%) or no wrinkles (26%). Elasticity indicators performed well for the great majority of them, while brightness proved to be their main concern.

Table 1: Study volunteers' socioeconomic and physical condition profile

Variable	Parameter	Frequencies	
		N	Percentage
Age	<25 years old	3	13%
	25-35 years old	6	26%
	35-45 years old	2	9%
	45-55 years old	5	22%
	>=55 years old	5	22%
	Non response	2	9%
Gender	Male	3	14%
	Female	19	86%
Schooling	Secondary School	1	4%
	Degree or higher	20	87%
	Non response	2	9%
Marital Status	Single	12	52%
	Married or cohabiting	9	39%
	Separated / divorced	1	4%
	Non response	1	4%
Professional Status	Student	9	41%
	Employeed	12	55%
	Retired / Pensioner	1	5%
Household Size	1 or 2 persons	6	26%
	3 or 4 persons	3	13%
	5 or more persons	6	26%
	Non response	8	35%
Montly Household Income	Any less de 500 €;	3	13%
	500 to 1000 €;	2	9%
	1000 to 3000 €;	12	52%
	3000€ or higher	4	17%
	Non response	2	9%
Body Mass Index	Healthy weight	14	61%
	Overweight	8	35%
	Obese	1	4%
Skin problems or diseases	Psoriasis	2	9%
	Rosacea	1	4%
	Acne	3	13%
	Atopic dermatitis	1	4%
	No skin problem	14	61%
	Other	2	9%

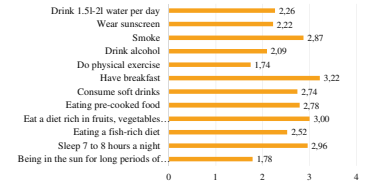


Figure 1: Score on healthy behaviours from the sample of volunteers (Positive behavious:1. Never or rarely; 2. Sometimes; 3. Often; 4.Always; Negative behaviours: 4. Never or rarely; 3. Sometimes; 2. Often; 1.Always))

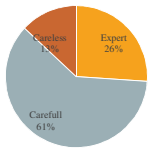


Figure 2: Skin care behavioural profile from the sample of volunteers

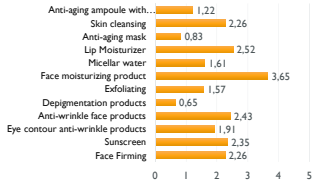


Figure 3: Score on habits of using facial cosmetic products from the sample of volunteers (0. Never; 1. 1 or 2 times a year; 2. 4 times a year; 3. 1 or 2 times a month; 4. 1 or 2 times a month or rarely; 5. Daily)

Results

The assessment of product efficacy was measured based on a questionnaire applied to the selected volunteer users. The twenty-two volunteers (one individual was unable to participate, due to logistic reasons) were asked to apply the product (**Figure 4**) at night, after face cleansing, for a two weeks' period.

None of the volunteers were excluded from the study, but 5 of them didn´t comply with the minimum of week of using. The end study results include data from seventeen users, five of them being placebo users.

The results obtained for non-placebo users in the assessments were presented in **Figure 4** for each of the attributes.

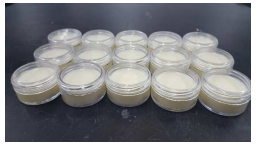


Figure 4: Image of the samples of the moisturizing cream

The product exhibits positive performance (score superior to 2 points) for all the attributes under assess. For products efficacy the highlight is for hydration and skin smoothness. For products attributes, ease application scores the maximum of 3.3 points. Different, wrinkles reduction and fragrance present less favourable results.

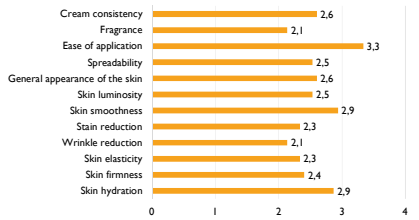


Figure 4: Results from consumers' satisfaction based on the self-assessment from the users' sample (0.Totally dissatisfied 1. dissatisfied; 2. Indifferent; 3. Satisfied; 4. Totally satisfied)

When asked about the willingness to buy the product **(Figure 5)** only one in four users respond negatively. The users willing to buy the product consider to do it at an average price of 10€ for a 50ml package **(Figure 6)**.

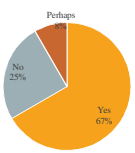


Figure 5:Willingness to buy the product tested of users

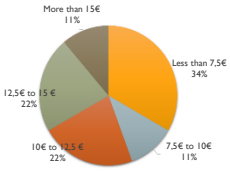


Figure 6:Willingness to pay for 50ml of product, in percentage of willing users

The main reasons for users willing to buy/recommending the product to a friend mentioned were the satisfactory results obtained, hydration performance and natural product as the determinant for that decision **(Figure 7)**. Four users respond negatively, mainly because of the strong fragrance of the preservative agent product.

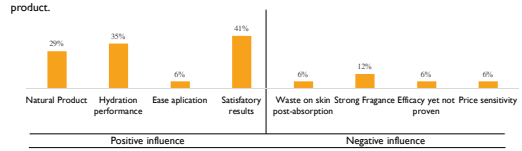


Figure 7: Determinant attributes on the recommendation/buying decision of the product, accordingly to the percentage of users

Conclusion

The present study analyzed the user self-assessment effectiveness of a cream formulation for daily moisturizing of the skin, with natural ingredients, including bee products, local plant material of the region of Trás-Os-Montes, and vegetable oils. A volunteer group of users answered a questionnaire and the evaluation of their answers contributed to inform us about the cream's effectiveness and areas for improvement in the formulation. The results obtained demonstrate that the product exhibits positive performance for all the attributes under assessment. When asked about the willingness to buy the product only one in four users responds negatively, mainly because of the strong fragrance of the preservative agent product.

Users willing to buy/recommending the product to a friend mention the satisfactory results obtained hydration performance and natural product as the determinant for that decision.

The next steps include the improvement of the cream formulation, namely, its consistency and fragrance, and expand the size of users' group and the experimentation period in order to have more robust results.

Plus, further analysis should be done to test for the influence of users' socioeconomic and physical condition, and lifestyle profile, and purchase intentions. Literature shows that consumers' product attitudes and shopping behaviour are influenced by their health and environmental consciousness (Kim and Seoch, 2009; Kim and Chung, 2011). Generally, people who strongly desire to maintain a youthful look and improve their appearance look for chemical-free personal care products. In the context of skin/hair care product purchases, consumers with high health consciousness may consider whether a product is safe for the skin and body; therefore they may be more seriously concerned with the types of ingredients used to make the product than the consumers with low health consciousness (Johri and Sahasakmontri, 1998).

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Acknowledgements

The authors would like to thank the Foundation for Science and Technology (FCT, Portugal) and the ERDF under the PT2020 program for financial support to CIMO (UID / AGR / 00690/2019).

The antimicrobial effects of the commercial lavender and incense essential oils and extracts as an ecofriendly cosmetics preservatives

Szymon Frąk,* Krzysztof Juś, Agata Stolarczyk

Department of Natuarl Sceince and Quality Assurance, Institute of Quality Science, Poznań University of Economics and Business, Al. Niepodległości 10, Poznań, Poland.

*szymon.frak@phd.ue.poznan.pl

Introduction

Essential oils have been known and used by humans for centuries. They are distinguished by a multi-component, unique form and strong action, including: antibacterial, antiviral and antifungal. They have a very wide range of applications in the pharmaceutical, cosmetic, chemical and even food industries, which is undoubtedly due to the fact that more and more consumers pay attention to the composition of products and the growing demand for natural and organic cosmetics. Especially the last ones – cosmetics use the benefits of aromatic plant substances.

This work indicates that essential oils and extracts can be treated as natural preservatives in ecological cosmetics in which one of the major problem is microbial purity ensuring. Contamination with microbes leads to the loss of physicochemical properties of cosmetics, and what is worse - they pose a health hazard to their users. In this context, it is worth paying special attention to the substances obtained from the aromatic plants like lavender and incense, which are known for theirs pro-health and physicochemical properties for a long time. Their addition, even in small amounts, can improve the safety of organic cosmetics, especially where the smell of these plants is not undesirable.

Materials

The key materials used in this study were: commercial, ECOSPA brand essential oils and extracts obtained from the lavender (*Lavandula Angustifolia*) and incense (*Boswellia Carteri*) plants. Their antimicrobial activity was determined against following strains of microorganisms: *Escherichia coli, Staphylococcus epidermidis, S. aureus, Pseudomonas aeruginosa, Bacillus subtilis and Micrococcus luteus and Candida albicans*. The microorganisms were incubated on TSA (trypticase soy agar), PCA (plate count agar) or SAB (sabouraud dexstrose agar with chloramphenicol) medium, depending on the nutritional needs of the microorganism.

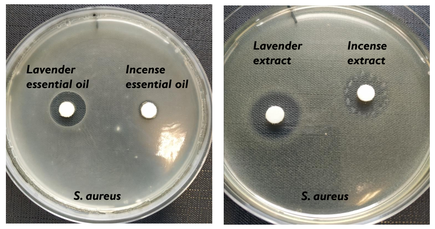
Methodology

In order to determine antimicrobial activity of the tested commercial lavender and incense essential oils and CO_2 extracts two methods were used:

1) Disc diffusion method was carried out by soaking the sterile discs (diameter 6 mm) in the test substances and placing them on agar plates previously inoculated with each strains of microorganisms. Two substances (in 100% concentration) were tested in one plate – comparative: lavender oil vs incense oil and lavender extract vs incense extract. The agar plates prepared in this way were incubated for 48 hours at the temperature of 37°C or 30°C depending on the tested microorganism. After incubation time the growth inhibition zone (in mm) around the discs were measured. Three parallel repetitions were performed.

2) The 96-well serial dilution method. The antimicrobial activity was tested in a concentration range from 10% to 0.08% (dissolved in DMSO). The simplified scheme of the tested plate is presented below. The one-day suspensions of the microorganisms were adjusted to an optical density of 0.5 on the McFarland scale. After 24 hours of incubation at 37°C or 30°C, basis of the absorbance measurement at a wavelength of 600 nm, the percentage inhibition of the microorganisms growth in the successive dilutions of the tested plant substances was determined.

An exemplary 96-well plate scheme for one microorganism and one repeat. The percentages represent the degree of growth inhibition relative to the control.Controls with DMSO, clear substances and medium were carried out on separate plates.



The agar plates with visible claer zones around discs

Results

This section presents the results of the conducted research. The table below shows the results from the serial dilution method. Based on the research, it was found that at the concentration > 1.25%, all tested plant substances inhibited the growth of microorganisms in at least 90%. It is worth to underline that the antimicrobial effect of tested plant essential oils and extracts depended mainly on strain of indicator microorganisms, less on the type of used plant substances.

Table I.The antimicrobial effect of selected plant substances shown as percentage of growth inhibition

Essential oil or extract concentration		microorganism						
		S. epidermidis	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*M. luteus*	*C. albicans*
Lavender essential oil	1,25%	98%	100%	100%	22%	96%	96%	100%
	0,30%	69%	90%	80%	4%	31%	89%	94%
	0,08%	14%	0%	79%	0%	5%	61%	18%
Lavender CO_2 extract	1,25%	100%	96%	100%	100%	97%	99%	100%
	0,30%	93%	63%	91%	15%	11%	12%	46%
	0,08%	18%	0%	91%	4%	0%	5%	0%
Incense essential oil	1,25%	17%	70%	80%	17%	100%	94%	100%
	0,30%	45%	0%	76%	4%	10%	26%	35%
	0,08%	11%	0%	70%	0%	4%	15%	17%
Incense CO_2 extract	1,25%	100%	100%	100%	30%	44%	94%	100%
	0,30%	100%	89%	91%	0%	0%	95%	40%
	0,08%	89%	74%	91%	0%	0%	88%	16%

P. aeruginosa and *E. coli* developed the strongest resistance to the tested substances, which is also confirmed by the disc diffusion method. Growth of *B. subtilis* was most effectively inhibited. The lavender CO_2 extract was the most universal and effective plant substances which inhibited each microorganisms in over 90% in concentration of 1.25%. However, in the case of three tested strains, it was incense extract that proved to be the stronger and inhibited the growth of microorganisms at the lowest concentration in over 70% (which does not correspond to the second method). There is no visible difference between the efficacy of lavender oil and extract in the ranges of the tested concentrations and microorganisms. However, in the case of substances obtained from the incense plant, the extract was much stronger. This observation is also confirmed by the second method of research (the results are presented in the chart below). This method also showed a generally much stronger effect of substances obtained from the lavender plant than from the incense plant.

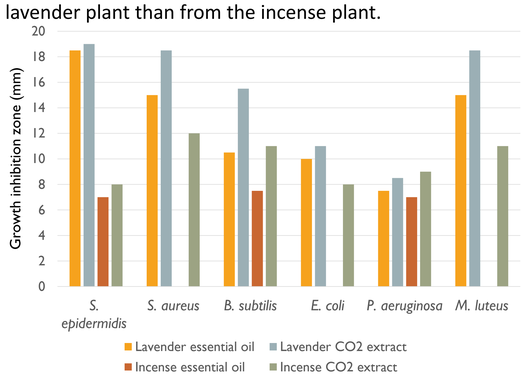


Chart I. Results of the disc diffusion method.

Conclusion and reccomendation

The obtained results confirm that aromatic plant substances from lavender and incense are effective agents that inhibit the growth of microorganisms and may also play an important role in microbial infection preventing in the cosmetics industry. The addition of plant extracts, especially popular in creams, can significantly reduce the risk of microbial infection which may cause the loss of cosmetic properties or even pose a threat to consumers health. Generally, lavender-derived substances are more versatile, but the incense extract is also promising. It is also important to indicate that the substances obtained by supercritical extraction are more effective than those obtained by distillation. This is especially important because of their more environmentally friendly extraction method.

UTILIZATION OF BEE PRODUCTS AND TRÁS-OS-MONTES AROMATIC PLANTS ON THE DEVELOPMENT OF COSMETIC FORMULATIONS

Sarra Jelidi[1], Olívia R. Pereira[2], Andrea F. Afonso[3], Luís Pedro[4], Susana M. Cardoso[3], Maria João Sousa[2]*

[1]School of Agriculture, Polytechnic Institute of Bragança, Campus of Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal; sarrajelidi7@gmail.com; [2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; oliviapereira@ipb.pt; joaos@ipb.pt; [3]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; andrea@ipb.pt; susanacardoso@ua.pt; [4]Centro de Biotecnologia Vegetal (CBV), C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal; lmpedro@ciencias.ulisboa.pt;
*Correspondence: joaos@ipb.pt; Tel.: +351-273-303-200

Introdução/Introduction

In this work we use, bee products, vegetable oils and plant material from the region of Trás-Os-Montes to develop a cosmetic formulation for the treatment and daily moister of the skin. As shown in the figure 1, the beeswax was chose in this work among the other bee products for its versatile properties and well-known characteristic's. *Salvia officinalis* and *Salvia elegans* were use as origin of active principles namely polyphenols, described as compounds with anti-inflammatory, and antiaging activity; a thyme plant, *Thymus zygis zygis*, endemic from this region, was used as a source of natural preservatives. Since cosmetic formulations includes oily compounds as hydrant, in this work two oils were use; olive oil and almond oil for their benefits for the skin, and also for their ability to dissolve fats and terpenes, and create an emulsion.

Figure 1: Elements intended for use in the formulation of the cream

Objetivos/Objectives

In this project, the main objective was to analyze plant material extracts to identify their bioactive compounds and their properties in order to incorporate them in several formulations. Once the desired qualitative criteria were reached, stability essays were performed and one of the formulations (the one that most closely matches the coveted criteria) was chosen and tested by several categories of individuals according to their skin type (mature or juvenile, oily or dry, etc.). Then, in order to evaluate the effectiveness of the product, post use questionnaire were developed.

Material e Métodos /Material and Methods

- **Extraction of *Thymus zygis* essential oil by Clevenger apparatus[1]**
- **Chemical analysis of *Thymus zygis* essential oil by Gas chromatography-mass spectrometry (GC-MS)[2]**
- **Microbial stability of *Thymus zygis* essential oil**

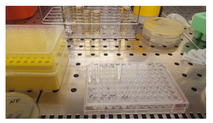


Figure 2 : Tests of inhibition of microbial growth (microdilution technique)

Figure 3 : Microbial contamination test (Agar diffusion)

- **Hydroalcoholic extraction of *Salvia elegans* and *Salvia officinalis* by solid-liquid extraction with an 96% ethanol solution**
- **Chemical analysis of *Salvia elegans* and *Salvia officinalis* hydroalcoholic extracts by (UHPLC -DAD-ESI/MSn)[3]**
- **Preparation of cream formulation**

Figure 4: Preparation of extracts

Table 1 : Cream ingredients

Ingredients	Function
Methylcellulose gel	Excipient
Olive oil	Oily material
Almond oil	
Beeswax	Source of natural active substances
Salvia elegans hydroalcoholic extract	
Salvia officinalis hydroalcoholic extract	
Tween 80	Emulsifiyng agent
Starch	Thickening agent
Thymus zygis oil	Preservative

- **Stability essays of the cream formulation**

Texture and consistency → Centrifugation test → Mechanical vibration test → pH determination → Spectrophotometric test

Light test → Viscosity test → Relative density → Extreme temperature → Accelerated testing

- **Questionnaire after use**

Resultados/Results

- **Chemical composition of *Thymus zygis* essential oil**

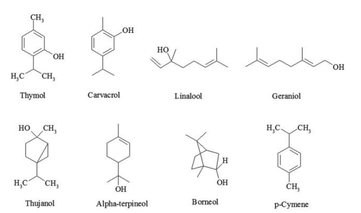


Figure 4 : Chemical structure of the main substances occurring in genus Thymus plants: thymol, carvacrol, linalool, geraniol, thujanol, α-terpineol, borneol, and p-cymene [4]

The chemical composition analysis also highlighted the presence of grouped components with a high percentage:

- Oxygen-containing monoterpenes 49,8%
- Monoterpenes hydrocarbons 49,6 %
- Carvacrol 43 %
- Cymene 24,10 %
- Trans-sabinene 15,8 %

- **Microbial stability of *Thymus zygis* essential oil**

Table 2 : Test of inhibition of microbial growth

	MIC	MBC
Escherichia coli	1.25 %	1,25 %
Pseudomonas sp	0,31 %	0,31 %
Staphylococcus aureus	< 0,031%	< 0,031%

Table 3 : Microbial contamination test

Oil concentration	Inhibition zone for *Candida albicans*
2,5%	9,5 mm
1,25%	6 mm
0,625%	2 mm
0,31%	-

- **Chemical analysis of *Salvia elegans* and *Salvia officinalis* hydroalcoholic extracts**

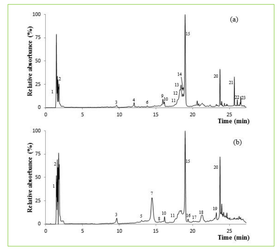


Figure 5 : Chromatographic representation of *S. officinalis* (a) and *S. elegans* (b) hydroethanolic extracts at 280 nm

(a) *S.officinalis* hydroethanolic extract :
Rosmarinic acid (peak 15)
Apigenin-O-glucuronide (peak 13)
Scutellarein-O-glucuronide (peak 9)
Luteolin-7-O-glucuronide (peak 10)

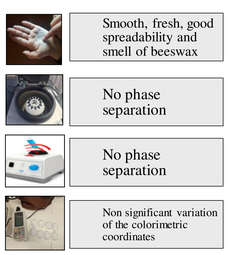
(b) *S.elegans* hydroethanolic extract :
Rosmarinic acid (peak 15)
Salvianolic acid K (peak 7 and 8)
Luteolin-7-O-glucuronide (peak 10)
Caffeic acid (peak 3)

- **Preparation of cream formulation**

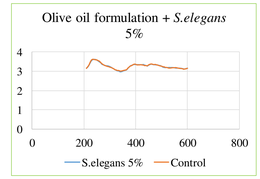
Table 4 : Types of formulations obtained

Olive oil formulation	Almond oil formulation
Control formulation	Control formulation
5% *Salvia elegans*	5% *Salvia elegans*
2,5% *Salvia elegans*	2,5% *Salvia elegans*
1,25% *Salvia elegans*	1,25% *Salvia elegans*
5% *Salvia officinalis*	5% *Salvia officinalis*
2,5% *Salvia officinalis*	2,5% *Salvia officinalis*
1,25% *Salvia officinalis*	1,25% *Salvia officinalis*

- **Stability essays of the cream formulation**



The domain in which the obtained pH values vary (6.5 ± 0.3 and 6.7 ± 0.2) is close to neutrality that may therefore have no effect on the ski microbiota

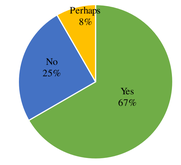


The olive oil samples containing the extracts of *Salvia elegans* have spectrophotometric profiles which are superimposed on those of control formulations, in particular that of 5%

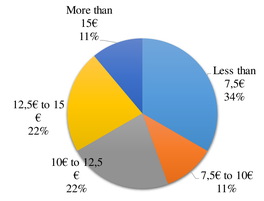
Figure 5 : Spectrophotometric profile of olive oil formulation + 5% *S.elegans* hydroalcoholic extract

According to this stability criterion this sample was selected to be tested

- **Questionnaire after use**



Willingness to buy the product, in percentage of users



Willingness to pay for 50ml of product, in percentage of willing users

Conclusões/Conclusions

- Physico-chemical stability of the different types of formulations
- Bactericidal power against *E.coli*, *Pseudomonas sp* and *S.aureus* and inhibitory power of *C.albicans* of Thyme oil
- Chemical analysis of *S.elegans* and *S.officinalis* hydroalcoholic extracts showed rich phenolic profiles
- The olive oil formulation containing 5% of *S.elegans* hydroalcoholic extract showed the best organoleptic criteria, hydrating power and photo stability profile
- Positive performance of the cream

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Agradecimentos/Acknowledgments

Os autores agradecem à Fundação para a Ciência e a Tecnologia (FCT, Portugal) e ao FEDER sob o Programa PT2020 pelo apoio financeiro ao CIMO (UID/AGR/00690/2019). The students co-authors want to thank their professors for their guidance and time invested in this study. Also, many thanks to IPB for the experimental work. Thanks to FCT (Portugal) and the ERDF under the PT2020 Program for financial support to CIMO (UIDB/00690/2020) and LAQV-REQUIMTE (UIDB/50006/2020). SMC acknowledges the research contract under the project AgroForWealth (CENTRO-01-0145-FEDER-000001.