ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 13:4 (2019) 355-362

records of natural products

Chemical Composition, Enantiomeric Distribution and AChE-BChE Activities of the Essential Oil of *Myrteola phylicoides* (Benth) Landrum from Ecuador

James Calva[*1], Jessica M. Castillo[1], Nicole Bec[2,4], Jorge Ramírez[1], José Miguel Andrade[1], Christian Larroque[2,3], and Chabaco Armijos[1]

[1]*Departamento de Química y Ciencias Exactas, Universidad Técnica Particular de Loja, San Cayetano Alto s/n, 1101608 Loja, Ecuador*

[2]*IRCM, Cancer Research Institute of Montpellier, INSERM, U1194, 34298 Montpellier, University of Montpellier France*

[3]*Supportive Care Unit, Montpellier regional Cancer Institut, ICM, 34298 Montpellier, France*

[4]*Institut for Regenerative Medicine and Biotherapy INSERM, U1183,34298 Montpellier, France*

(Received September 11, 2018; Revised December 07, 2018; Accepted December 08, 2018)

Abstract: The volatile constituents of the essential oil (EO) of *Myrteola phylicoides* (Benth) Landrum, from Ecuador, extracted by steam distillation have been analyzed. A total of 37 compounds, representing 90.30% the total essential oil sample were identified. Monoterpenes hydrocarbons (53.06%) and sesquiterpene hydrocarbons (35.24%) were the principal groups of compounds. The major components were identified as *α*-pinene (30.94%), (*E*)-caryophyllene (21.93%), *β*-pinene (14.45%) and *α*-humulene (9.56%). The essential oil of *M. phylicoides* showed weak *in vitro* activity against AChE inhibition with IC_{50} value 60.8 µg/mL and a low BChE activity with IC_{50} value >250 µg/mL. This is the first report on the chemical composition of the essential oil of this species.

Keywords: *Myrteola phylicoides*; essential oil; AChE; BChE.

1. Introduction

The research of new products of natural origin has contributed significantly in the discovery of new substances with therapeutic properties [1,2], due to the diverse range of secondary metabolites that they possess and the wide range of pharmacological activities that they show [3,4]. In this sense, Ecuador stands out for having large plant biodiversity [5,6,7] and because most of its plants are unexplored relative to their pharmacological potential.

The Myrtaceae is a large family with approximately 140 genera and approximately 3500-5800 species [8] distributed mainly in the humid tropics, especially in South America, Australia and tropical Asia. These plants are characterized by having fibrous bark, mostly with a lower ovary, opposite leaves and possessing aromatic essential oils [9]. In Ecuador it is distributed in the provinces of Azuay, Loja, Napo, Morona Santiago and Zamora Chinchipe [10]. For a long time, several species of this family have been used by indigenous communities of Ecuador in the preparation of traditional foods and beverages such as leaves of *Myrcianthes fragrans* (Sw.) McVaugh and *Myrcianthes hallii* (O. Berg) McVaugh that are aromatic additives of colada morada, which is typically drunk in the Day of the Dead or All Soul's Day

[*] Corresponding author: E-Mail: jwcalva@utpl.edu.ec ; Phone: +593-7370-1444 (ext. 3049)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products © July-August 2019 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.112.18.09.893

[11,12]. Likewise, the leaves from *M. phylicoides* are used by the "Saraguros ethnic group" in the Andean region of south Ecuador for the treatment fever, cold and "mal aire" (a supernatural disease caused by strong winds) [13,14]. De la Torre et al. (2008) reported that the aqueous extract of the *M. phylicoides* mixed with water or milk is used to treat measles [15]. Previous studies have described antiradical, antioxidant and antiinflammatory properties activity, the total phenolic content and the total content of anthocyanins, flavonols, antioxidants and anti-inflammatories and phenolic acids [16, 17]. Among some important data of the *Myrteola* genus has been described the inhibition of the proliferation of cancer cells of *Myrteola nummularia* [18].

Nevertheless, no phytochemical and pharmacological investigation exist which indicate the presence of bioactive compounds in this specie or in their essential oil. Therefore, we considered it interesting to investigate the chemical composition, enantiomeric distribution of the essential oil of *M. phylicoides.*

In addition, we have previously documented the effect of equatorian plants extract on acetyl- and butyryl-cholinesterases. These activities are of particular interest since theses enzymes play a key pivotal role in biological processes affected in neurological disorders as well as inflammatory context. In order to complete our data we present data on the effect of the essential oil of *M. phylicoides* on these enzymes [19].

This is the first report of the chemical composition, enantiomeric distribution and AChE-BChE activities of the essential oil of *M. phylicoides* (Benth) Landrum.

2. Materials and Methods

2.1. Plant Material and Preparation of the Essential Oil

The aerial parts of *M. phylicoides* were collected in "Patunadana" (9590410N, 17692602E) in San Lucas, Saraguro region in Loja Province, Ecuador on November 2017. The plant species was identified by Dr. Fani Tinitana of the HUTPL Herbarium. The scientific name was based on the Catalogue of the Vascular Plants of Ecuador [10]. The plant was collected under permission n. 001-IC-FLO-DBAP-VS-DRLZCH-MA of the Ministry of Environment of Ecuador (MAE) and a voucher specimen of *Myrteola phylicoides* is conserved in the Herbarium HUTPL of the *Universidad Técnica Particular de Loja* under the code PPN-my-006.

The essential oil was hydro-distillated of fresh leaves in a Clevenger-type apparatus for four hours. Subsequently, the essential oil was tagged and stored in a brown vial at 4ºC until analysis.

2.2. Physical Properties of the Essential Oil

The physical properties of EO as: relative density (d^{20}), refractive index (n^{20}) and optical activity $[\alpha]_D^{20}$ were determined as the means of three different experiments done at 20 ºC, using a pycnometer (1 mL), a refractometer (model ABBE), and a polarimeter Hanon P 810, for relative density, refractive index and optical activity respectively.

2.3 Gas Chromatography Coupled Mass Spectrometry Analysis

The GC-MS analysis of the essential oil composition was performed using an Agilent Chromatograph (6890N series), coupled to a mass spectrometer-detector (Agilent 5973 series). The spectrometer, controlled by the data system MSD-Chemstation D.01.00 SP1, operated in the electron impact (EI) mode (electron energy at 70 eV); electron multiplier 1600 V; scan rate: 2 scan/s; mass range: 40-350 *m/z*. The GC column was a non-polar capillary column, DB-5MS 5%-phenyl-methylpolysiloxane stationary phase (30 m × 0.25 mm, 0.25 μm film thickness, Agilent, USA); helium was the carrier gas at a flow rate of 1.0 mL/min in constant flow mode; the detector and injector temperatures were set at 250 ºC.

The injector operated in split mode (40:1). The GC oven temperature was set at 60 ºC for 5 min, then increased to 165 ºC, with a gradient rate of 3 ºC/min, followed by an increase to 250 ºC with a gradient of 15 ºC/min and held for 10 minutes. The ion source temperature was 250 ºC. Samples were dissolved in dichloromethane (Fisher Scientific, relation 1:100 (v/v) and 1 μL of the solution was injected.

The constituents of the essential oil were identified, by comparing their Linear Retention Indices calculated (LRIcal) and Linear Retention Indices-Mass Spectra (LRI-MS) data present in literature [20].

The retention indices were determined according to Van Den Dool and Kratz [21]. The retention indices were calculated using a homologous series of hydrocarbons C10-C25 (from Fluka, purity 99%), which were analyzed by GC immediately after oil samples, under the same conditions. The identification was considered as acceptable in a range of ±14 units of LRI values, according to the injection of some standard compounds belonging to the different terpenic families.

2.4. Gas Chromatography Coupled Flame Ionization Detector Analysis

Quantitative analysis of the essential oil was performed on an Agilent Technologies chromatograph (model 6890N series), using a flame ionization detector (FID). The percentage composition of the oil was determinate by correlating GC peak areas to the total chromatogram, with applying any correction factor, but normalizing with nonane as an internal standard. The analytical parameters were the same as the GC-MS analysis.

2.5 Enantioselective Distribution

Enantioselective GC-MS analysis was performed using the following parameters: the MS operated in electron impact ionization mode at 70 eV, operated with a mass range of *m/z* 40-350 full scan mode. The ion source temperature was set at 200 ºC. Helium was the carrier gas at a flow rate of 1.0 mL/min. The injector operated in split mode (40:1) at 200 ºC. The oven thermal program was set at 50 ºC for 2 min, and then increased to 220 ºC, with a gradient rate of 2 ºC/min and held for 2.0 min. A chiral capillary column based on cyclodextrin diethyl tertbutylsilyl-β-CDX (25m × 0.25mm dc × 0.25mm df) from Mega (Legnano, MI, Italy) was used. The essential oil samples were dissolved in dichloromethane (Fisher Scientific, relation 1:100 (v/v) and 1 µL of the solution was injected.

2.6 Cholinesterase Inhibition Test

Cholinesterase (ChE) activities were assayed following a colorimetric protocol adapted from Ellman et al. [22] ChEs efficiently catalyze the hydrolysis of acetylthiocholine (ATCh), the sulfur analog of the natural substrate of these enzymes. Upon hydrolysis, this substrate analog produces acetate ion and thiocholine. Thiocholine, in the presence of the highly reactive dithiobisnitrobenzoate (DTNB) ion, generates a yellow color, which can be quantitatively monitored by spectrophotometric absorption at 412 nm. All reagents were obtained from the Sigma-Aldrich trading house. A typical 200 µL inhibition assay volume contained phosphate buffered saline solution (pH 7.4), DTNB (1.5 mM), test sample in DMSO (1% v/v final). Both acetylcholinesterase from *Electrophorus electricus* (Type V-S, lyophilized powder, 744 U/mg solid, 1272 U/mg protein) and butyrylcholinesterase from equine serum (lyophilized powder, ≥900 unit's/mg protein) were dissolved in Phosphate Buffered Saline (PBS) pH 7.4 and used at 25 mU/mL for the assay. After 10 min of pre-incubation, the substrate acetylthiocholine iodide (1.5 mM) was added to start the reaction. During 1 h of incubation, 96-well microtiter plates were read on a PherastarFS (BMG Labtech) detection system. All measurements were made in triplicate. When possible, the IC_{50} values were calculated using the GNUPLOT package on line (www.ic50.tk, www.gnuplot.info). Donepezil was used as reference ChE inhibitor with an IC_{50} = 100 nM for AChE and 8500 nM for BChE. In this assay, we did not exclude the possibility of false-positive inhibition results previously described for high concentrations (>100 µg/mL) of amine or aldehyde compounds [23, 24].

3. Results and Discussion

3.1 Physical Properties

The clear pale yellow essential oil was obtained in 0.15±0.02 % yield. Three physical properties were determined: refractive index (n = 1.49±0.002), relative density (d=0.91± 0.012 g/L), and optical rotation ($[\alpha]_D^{20}$ -5.32±0.137 in CH_2Cl_2, c = 10.0).

3.2 Chemical Composition

This is the first report on the chemical composition of the essential oil of *M. phylicoides*. The chemical composition of *M. phylicoides* essential oil is compiled in Table 1.

Table 1. Chemical composition of the essential oil from *Myrteola phylicoides*

Compound	**LRI^{cal}**	**LRI^{lit}**	**%**
α-Thujene	925	924	0.09
α-Pinene	934	931	30.94
Camphene	948	946	0.63
β-Pinene	979	974	14.45
Myrcene	989	988	2.86
α-Phellandrene	1006	1002	t
α-Terpinene	1016	1014	0.11
p-Cymene	1024	1020	0.08
Limonene	1029	1024	3.16
1,8-Cineole	1035	1026	0.78
γ- Terpinene	1057	1054	0.33
Terpinolene	1084	1086	0.40
Linalool	1101	1095	0.38
n-Nonanal	1105	1110	0.09
exo-Fenchol	1119	1118	0.07
3-Thujanol	1172	1164	t
Terpinen-4-ol	1181	1174	0.09
endo-Fenchyl acetate	1218	1218	t
(*E*)-Anethole	1284	1282	t
2-Undecanone	1293	1293	0.06
Myrtenyl acetate	1323	1324	0.03
Linalool isobutanoate	1359	1373	t
Isoledene	1374	1374	0.33
Sibirene	1405	1400	0.14
(E)-Caryophyllene	1422	1417	21.93
Longifolene	1426	1407	0.09
Aromadendrene	1437	1439	1.22
Myltayl-4(12)-ene	1444	1445	0.13
α-Humulene	1456	1452	9.56
Himachalene	1459	1449	0.09
γ-Gurjunene	1470	1475	0.16
γ-Muurolene	1473	1478	t
Viridiflorene	1488	1496	0.78
δ-Amorphene	1501	1511	0.66
γ- Cadinene	1510	1513	0.08
δ-Cadinene	1516	1522	0.16
α-Caracolene	1538	1544	0.47
	Monoterpenes hydrocarbons		53.1
	Oxygenated monoterpenes		1.3
	Sesquiterpenes hydrocarbons		35.2
	Others		0.7
	Total		**90.3**

LRI^{cal} Linear retention indices calculated in reference of a homologous series of n-alkanes on DB-5MS capillary column; LRI^{lit} Linear Retention Indices from the literature [20]; RSD% below 5%; trace (t) <0.03%. RI = retention index; MS = mass spectroscopy.

The essential oil was analyzed by GC-MS and GC-FID. Thirty seven constituents were identified, which corresponding to 90.3% of all the oil, with the major constituents in the sample analyzed were identified as *α*-pinene (30.9%), *(E)*-caryophyllene (21.9%), *β*-pinene (14.5%), *α*-humulene (9.6%) and limonene (3.2%) (Table1). Monoterpenes hydrocarbons (53.1%) and sesquiterpene hydrocarbons (35.2%)

were the principal groups of compounds, and oxygenated monoterpenes (1.3%) were the minor groups present in the essential oil.

Like in the EO of *M. phylicoides* we found high content of *α*-pinene and *β*-pinene compounds in other genera of Myrteola such as: *Blepharocalyx salicifolius* from Bolivia, 34% of *β*-pinene and 17% *α*-pinene are reported, likewise in *Eugenia rotundifolia* the percentaje of *α*-pinene is 15.8% [25, 26]. Precedent studies have shown that the *α*-pinene may have anti-inflammatory effects in human chondrocytes, thus exhibiting potential antiosteoarthritic activity [27] and antimicrobial potential [28]. The *β*-pinene shown phytochemicals properties against gram-positive bacteria [29]. In terms of applications, *α*- and *β*-pinene are most commonly used in solvents such as turpentine (a cleanin solvent), as well as in the fragrances industry as building blocks for artificial odorants [30].

(*E*)-Caryophyllene is used in spice blends, citrus flavors, soaps, detergents, creams and lotions, and in a variety of food products and beverages. (*E*)-Caryophyllene is also known for its anti-inflammatory and local anesthetic properties [31].

The *α*-humulene acts as an antibacterial agent and has anti-cancer and anti-inflammatory properties. In small quantities, it has been shown to kill the *S. aureus* bacteria. A study showed that humulene, especially when acting in concert with other terpenes and cannabinoids, killed cancer cells. The most recent studies concluded that *α*-humulene was as effective of an anti-inflammatory as the steroidal drug dexamethasone. Further still, *α*-humulene is frequently invoked as an appetite suppressant, which may lead to more widespread use in the future [32].

3.3. Enantioselective GC-MS Analysis

The enantiomeric distribution and enantiomeric excesses (*e.e.*) of essential oil were performed by enantioselective GC-MS on a cyclodextrine-based chiral stationary phase. Four couples of chiral monoterpenoids were detected (Table 2). These same compounds were previously reported in *L. mutica* essential oil [33]. The LRI of (+)-*β*-pinene, (-)-*β*-pinene were similar 962, 957 and 962, 965 respectively to reported for *L. mutica* [34] and *N. dissecta* [35], the *(+)-β*-pinene enantiomer were highly toxic to *Candida albicans* [36]

Table 2. Enantiomeric analysis of the components of *Myrteola phylicoides* essential oil

Compound	**LRI**^{cal}	**Enantiomeric distribution %**	***e.e.* (%)**
(+)-*β*-pinene	961	99.79	99.57
(-)-*β*-pinene	969	0.21	
(+)-linalool	1198	27.85	44.31
(-)-linalool	1209	72.15	
(+)-terpinen-4-ol	1269	56.00	12.01
(-)-terpinen-4-ol	1272	44.00	
(+)-*α*-terpineol	1312	72.65	45.31
(-)-*α*-terpineol	1325	27.35	

LRI^{cal} Linear retention indices calculated in reference of a homologous series of n-alkanes on MEGA-DEX DET Beta capillary column; *e.e.* enantiomeric excess.

Three couples and one enantiomerically pure chiral monoterpenoide were detected and baseline separated. (+)-*β*-pinene was detected as enantiomerically pure compound while (-)-linalool and (+)-*α*-terpineol were present in mixture with their enantiomers but with a very high *ee* value. In contrast, the enantiomeric excess of (+)-terpinen-4-ol was almost racemic. These results further confirm that secondary metabolites can be present in plants as enantiomeric mixtures.

3.4. Cholinesterase Inhibition Test

The essential oil showed a weak inhibitory activity against AChE and BChE with IC_{50} concentrations 60.8 µg/mL and >250 µg/mL, respectively. The study by Dohi et al., showed that the activity of α-pinene, the main component in some essential oils, does not contribute much to the activities against AChE [36]. For this reason, it is necessary to carry out new studies that allow to determine if any isolated compound of the essential oil of *M. phylicoides* present a marked activity on the enzymatic systems studied, this could also contribute to know the anti-AChE symbiotic effect that can exert each compound.

Acknowledgments

The authors thank to the Universidad Técnica Particular de Loja, Ecuador, for financial support. C. Larroque was a recipient of the ecuadorian SENESCYT-PROMETEO program.

ORCID
James Calva: 0000-0003-0608-8990
Jessica M. Castillo: 0000-0003-0047-3150
Nicole Bec: 0000-0001-7608-2434
Jorge Ramirez: 0000-0002-8839-7457
Jose Miguel Andrade: 0000-0003-0181-3065
Christian Larroque: 0000-0003-3833-0606
Chabaco Armijos: 0000-0002-3252-9932

References

[1] I. Ahmad and A.Z. Beg (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, *J. Ethnopharmacol*. **74(2)**, 113-123.

[2] Jr. Viegas, V.d.S. Bolzani and E.J. Barreiro (2006). Os produtos naturais e a química medicinal moderna, *Quim. Nova*, **29**, 326-337.

[3] E. Christaki, E. Bonos, I. Giannenas and P. Florou-Paneri (2012). Aromatic plants as a source of bioactive compounds, *Agriculture*. **2,** 228-243.

[4] E. Pichersky and D.R. Gang (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective, *Trends Plant Sci*. **5(10)**, 439-445.

[5] V. Tene, O. Malagon, P.V. Finzi, G. Vidari, C. Armijos and T. Zaragoza (2007). An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador, *J. Ethnopharmacol*. **111(1)**, 63-81.

[6] M. Ríos, M.J. Koziol, H.B. Pedersen and G. Granda (2007). Plantas útiles del Ecuador: aplicaciones, retos y perspectivas/Useful plants of Ecuador: applications, challenges, and perspectives. Quito: Ediciones Abya-Yala.

[7] P. Sklenár and P.M. Ramsay (2001). Diversity of zonal paramo plant communities in Ecuador, *Divers. Distrib*. **7(3)**, 113-124.

[8] N.S. Frauches, T.O. do Amaral, C.B.D. Largueza and A.J. Teodoro (2016). Brazilian myrtaceae fruits: a review of anticancer proprieties, *BJPR*. **12(1),** 1-15

[9] R. Govaerts, M. Sobral, P. Ashton, F. Barrie, B.K. Holst, L.L. Landrum and L.H. Soares-Silva (2008). World checklist of Myrtaceae, *RBGK*.

[10] P.M. Jørgensen, S. Léon-Yánez and M.B. Garden (1999). Catalogue of the vascular plants of Ecuador: Missouri Botanical Garden Press.

[11] C. Armijos, E. Valarezo, L. Cartuche, T. Zaragoza, P.V. Finzi, G.G. Mellerio and G. Vidari (2018). Chemical composition and antimicrobial activity of *Myrcianthes fragrans* essential oil, a natural aromatizer of the traditional ecuadorian beverage colada morada, *J. Ethnopharmacol*. **225**, 319-326.

[12] P. Chavez Carvajal, E. Coppo, A. Di Lorenzo, D. Gozzini, F. Bracco, G. Zanoni and M. Daglia (2016). Chemical characterization and in vitro antibacterial activity of *Myrcianthes hallii* (O. Berg) McVaugh (Myrtaceae), a traditional plant growing in Ecuador, *Materials* **9(6),** 454.

[13] J.M. Andrade, H. Lucero Mosquera and C. Armijos (2017). Ethnobotany of indigenous Saraguros: medicinal plants used by community healers "Hampiyachakkuna" in the San Lucas parish, southern Ecuador, *BioMed. Res. Int*. **2017**: 9343724.

[14] C. Armijos, I. Cota and S. González (2014). Traditional medicine applied by the Saraguro yachakkuna: a preliminary approach to the use of sacred and psychoactive plant species in the southern region of Ecuador, *J. Ethnobiol. Ethnomed*. **10(1),** 26, 12 pages.

[15] L. De la Torre, H. Navarrete, P. Muriel, M.J. Macía and H. Balslev (2008). Enciclopedia de las plantas útiles del Ecuador. Herbario QCA de la Escuela de Ciencias Biolgicas de la Pontificia Universidad Católica del Ecuador & Herbario AAU del Departamento de Ciencias Biológicas de la Universidad de Aarhus.

[16] K.A. Reynertson, H. Yang, B. Jiang, M.J. Basile and E.J. Kennelly (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits, *Food Chem*. **109(4),** 883-890.

[17] L.M. De Oliveira, A. Porte, R.L. de Oliveira Godoy, M. da Costa Souza, S. Pacheco, M.C.P. de Araujo Santiago and R.G. Borguini (2017). Chemical characterization of *Myrciaria floribunda* (H. West ex Willd) fruit, *Food Chem*. **248**, 247-252.

[18] S. Flis, Z. Jastrzebski, J. Namiesnik, P. Arancibia-Avila, F. Toledo and H. Leontowicz (2012). Evaluation of inhibition of cancer cell proliferation in vitro with different berries and correlation with their antioxidant levels by advanced analytical methods, *J. Pharm. Biomed. Anal*. **62**, 68–78.

[19] C. Armijos, G. Gilardoni, L. Amay, A. Lozano, F. Bracco, J. Ramirez and G. Vidari (2016). Phytochemical and ethnomedicinal study of Huperzia species used in the traditional medicine of Saraguros in southern Ecuador; AChE and MAO inhibitory activity, *J. Ethnopharmacol*. **193**, 546-554.

[20] R.P. Adams (2009). Identification of essential oil components by gas chromatography/mass spectrometry (4th. Ed). USA: Allured Publishing Corporation.

[21] H. Van den Dool and P.D. Kratz (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography, *J. Chromatogr. A*. **11**, 463-471.

[22] G.L. Ellman, K.D. Courtney, Jr. Andres and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7(2),** 88-95.

[23] I.K. Rhee, R.M. Van Rijn and R. Verpoorte (2003). Qualitative determination of false positive effects in theacetylcholinesterase assays using thin layer chromatography, *Phytochem. Anal*. **14**, 127–131.

[24] I.K. Rhee, M. van de Meent, K. Ingkaninan and R. Verpoorte (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining, *J. Chromatogr A*. **915**, 217–223.

[25] F.-X. Garneau, G.J. Collin, F.-I. Jean, H. Gagnon and J.B.L. Arze (2013). Essential oils from Bolivia. XIII. Myrtaceae: *Blepharocalyx salicifolius* (Kunth.) O. Berg, *J. Essent. Oil Res*. **25(3),** 166-170.

[26] M.F.d.S. Ramos, S.d.S. Monteiro, V.P. da Silva, M.J. Nakamura and A.C. Siani (2010). Essential oils from myrtaceae species of the brazilian southeastern maritime forest (Restinga), *J. Essent. Oil Res.* **22(2),** 109-113.

[27] A. T. Rufino, M. Ribeiro, F. Judas, L. Salgueiro, M. C. Lopes, C. Cavaleiro & A.F. Mendes (2014). Anti-inflammatory and chondroprotective activity of (+)-α-pinene: structural and enantiomeric selectivity, *J. Nat. Prod*. **77(2),** 264-269.

[28] A. M. Leite, E. D. O. Lima, E. L. D. Souza, M. D. F. F. M. Diniz, V. N. Trajano and I. A. D. Medeiros (2007). Inhibitory effect of beta-pinene, alpha-pinene and eugenol on the growth of potential infectious endocarditis causing gram-positive bacteria, *Rev. Bras. Cienc. Farm*. **43(1),** 121-126.

[29] L. de Sousa Eduardo, T. C. Farias, S. B. Ferreira, P. B. Ferreira, Z. N. Lima and S. B. Ferreira (2018). Antibacterial activity and time-kill kinetics of positive enantiomer of α-pinene against strains of *Staphylococcus aureus* and *Escherichia coli*, *Curr. Top. Med. Chem*. **18(11),** 917-924.

[30] A.C. Silva, P.M. Lopes, M.M. Azevedo, D.C. Costa, C.S. Alviano and D.S. Alviano (2012). Biological activities of α-pinene and β-pinene enantiomers, *Molecules* **17 (6),** 6305-16.

[31] R.M. Montanari, L.C. Barbosa, A.J. Demuner, C.J. Silva, L.S. Carvalho and N.J. Andrade (2011). Chemical composition and antibacterial activity of essential oils from Verbenaceae species: alternative sources of (E)-caryophyllene and germacrene-D, *Quim. Nova.* **34(9),** 1550-1555.

[32] A.P. Rogerio, E.L. Andrade, D.F. Leite, C.P. Figueiredo and J.B. Calixto (2009). Preventive and therapeutic anti- inflammatory properties of the sesquiterpene α- humulene in experimental airways allergic inflammation, *Br. J. Pharmacol*. **158(4),** 1074-1087.

[33] J. Ramírez, G. Gilardoni, M. Jácome, J. Montesinos, M. Rodolfi, M.L. Guglielminetti and G. Vidari (2017). Chemical composition, enantiomeric analysis, AEDA sensorial evaluation and antifungal activity of the essential oil from the ecuadorian plant *Lepechinia mutica* Benth (Lamiaceae), *Chem. Biodivers*. **14(12).** https://doi.org/10.1002/cbdv.201700292

[34] J. Ramírez, G. Gilardoni, E. Ramón, S. Tosi, A.M. Picco, C. Bicchi and G. Vidari (2018). Phytochemical study of the ecuadorian species *Lepechinia mutica* (Benth.) Epling and high antifungal activity of carnosol against *Pyricularia oryzae*, *Pharmaceuticals*, **11(2),** pii: E33. doi: 10.3390/ph11020033.

[35] J. Calva, N. Bec, G. Gilardoni, C. Larroque, L. Cartuche, C. Bicchi and J.V. Montesinos (2017). Acorenone B: AChE and BChE inhibitor as a major compound of the essential oil distilled from the ecuadorian species *Niphogeton dissecta* (Benth.) JF Macbr, *Pharmaceuticals*. **10(4),** pii: E84. doi: 10.3390/ph10040084.

[36] S. Dohi, M. Terasaki and M. Makino (2009). Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils, *J. Agric. Food Chem*. **57(10),** 4313-4318.