Artículo original

Antioxidant activitiy and chemical composition of the essential oil of *Minthostachys mollis* (Benth.) Griseb from Ecuador.

Actividad antioxidante y composición química del aceite esencial de *Minthostachys mollis* (Benth.) Griseb de Ecuador.

Toaquiza-Aguagallo Cecilia[1], Cando-Brito Verónica[1], †Rojas-Fermín Luis[2], Pérez-Colmenares Alida[2]*, Aparicio-Zambrano Rosa[2], Obregón-Díaz Ysbelia[2].

[1]Faculty of Sciences, Chimborazo Higher Polytechnic School. Riobamba, C.P 060106, Ecuador. [2]Research Institute "Dr. Alfredo Nicolás Usubillaga del Hierro", Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, C.P. 5101, Venezuela.

Recibido: enero de 2022–Aceptado: abril de 2022

RESUMEN

El aceite esencial de las flores y hojas de *Minthostachys mollis* (Benth.) Griseb recolectada en la Provincia de Chimborazo, Ecuador, fue obtenido por hidrodestilación y su composición química fue determinada por cromatografía de gases acoplada a espectrometría de masas (**CG/EM**). Doce componentes fueron identificados, lo cual representó el 98,86% y 99,99% del total de los aceites de flores y hojas, respectivamente. Los compuestos mayoritarios del aceite esencial de las flores fueron la pulegona (45,15%), mentona (37,66%) y neomentol (6,03%), mientras que para el aceite esencial de las hojas fueron la mentona (46,58%), pulegona (18,76%), neomentol (12,65%) y acetato de neomentilo (9,91%). La actividad antioxidante fue determinada por el método del **DPPH** (2,-difenil-1-picrilhidrazilo). La concentración inhibitoria media (**CI_{50}**) fue de 32,73 µg/mL y 15,40 µg/mL para el aceite esencial de flores y hojas, estos resultados indican una actividad antioxidante elevada para ambos aceites.

PALABRAS CLAVE

Minthostachys mollis, aceites esenciales, pulegona, mentona, **DPPH**.

ABSTRACT

The essential oil from the flowers and leaves of Minthostachys mollis (Benth.) Griseb collected in the Province of Chimborazo, Ecuador was obtained by hydrodistillation and its composition was determined by **GC** and **GC/MS**. Twelve components were identified in the flowers and leaves oil, which represent 98.86% and 99.99% of the total oil, respectively. The major components of the flowers oil were pulegone (45.19%), menthone (37.66%) and neomenthol (6.03%) and the major components of the leaves oil were, menthone (46.58%), pulegone (18.76%), neomenthol (12.65%) and neomenthyl acetate (9.91%). The antioxidant activity was determined by interaction with the stable free radical 2,2-diphenyl-1-picrylhydrazyl. The **IC_{50}** of the essential oil of flowers and leaves were 32.73 µg/mL, and 15.40 µg/mL, respectively. These results indicated powerful antioxidant activity for both oils.

Correspondencia al autor: alidaperezc@gmail.com

KEY WORDS

Minthostachys mollis, essentials oils, pulegone, menthone, **DPPH**.

INTRODUCTION

The genus *Minthostachys* Griseb. (Lamiaceae) is found in middle elevations along the Andes, from Venezuela to Argentina. This genus includes 17 species according to some authors. It is of great ethnobotanical, pharmacological and commercial interest because of the essential oils found in the plants. It does not only find use, as a condiment or tea, in the traditional cuisine of the Andes, but is one of the most important plants in the folk medicine of the area. On the basis of this observation, it receives growing attention from modern pharmacology and medicine, as plant decoctions and extracted essential oils are tested for pharmacological effects [1-3].

From the early 16th century the folklore medicinal use of the *Minthostachys* genus has been reported for the treatment of several health-disorders such as headache, cold and flu, respiratory illnesses (asthma, bronchitis, cough), digestive disorders (indigestion, carminative, stomach-ache, diarrhea, colics), muscle spasms, rheumatism, impotence and amenorrhea. Other traditional uses of *Minthostachys* include biopesticides (antimycotic and antiparasitic, against flea infestations) and for the protection of stored potato and oca tubers from aphids and pests. In recent years, there have been numerous research studies on *Minthostachys* oils to provide scientific evidences on their medicinal properties [4-7].

Minthostachys mollis (Benth.) Griseb is restricted to the Andes of Ecuador, Venezuela, Colombia, Perú and Bolivia. It´s known by various popular names and has diferent medicinal uses. In Ecuador, *M. mollis* is known as Muña and is prepared as tea and used to treat infectious diseases of children, as well as for rheumatism. Traditionally it is used for the treatment of cough, bronchitis, stomach ulcer, gastritis, stomach and intestinal spasm, cold, headache, intestinal parasitism and as digestive. It is important to highlight that currently the trend of consumers is inclined to the consumption of foods free of synthetic products, so it is interesting to study native plants in this region to recommend its application as an additive for industrial purposes [8-10]. In the present study, the composition chemical and in vitro antioxidant activities of essentials oils of flowers and leaves of *Minthostachys mollis* collected of the Province of Chimborazo-Ecuador are reported.

MATERIALS AND METHODS

Plant material: Flowers and leaves of *Minthostachys mollis* (Benth.) Griseb were collected in January 2018 at Llucud in the Province of Chimborazo, Ecuador, at 3200 m above sea level, 1°43'22"S, and 78°33’15” W. A voucher specimen was deposited in the Herbarium Chimborazo Higher Polytechnic School. The botanical identification was made by Biologist Ricardo Zambrano from Faculty of Biological Sciences of the Central University of Ecuador.

Extraction and analysis of the essential oil: The fresh plant materials were separated into flowers (474.0 g) and leaves (788.0 g) cut into small pieces and submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. A volume of 0.45 mL and 0.75 mL of essential oil were obtained, respectively. The composition of the essential oils was determined by comparing of the mass spectrum of each compound with Wiley **GC/MS** library data and also from retention index (**RI**) data.

Gas chromatography (GC): **GC** analyses were performed using a Perkin-Elmer Autosystem gas chromatograph equipped with a FID detector and data-handling system. A 5% phenylmethylpolysiloxane fused-silica capillary column was used (30 m x 0.25 mm i.d., film thickness 0.25 m; HP-5, Hewlett-Packard, CA, USA). The oven temperature was programmed from 60°C to 260°C at 4°C/min. The injector and detector temperatures were 200°C and 280°C, respectively. The carrier gas was helium at 0.8 mL/min. The sample (1.0 µL) was injected using a split ratio of 10:1. Retention indices were calculated with reference to C8-C24 n-alkanes.

The percentage composition of the oil was calculated by the normalization method from the GC peak areas.

Gas chromatography–mass spectrometry (GC-MS): **GC-MS** analyses were carried out on a Model 5973 Hewlett-Packard **GC-MS** system fitted with a HP- 5MS fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm, Hewlett-Packard). The oven temperature program was the same as that used for the HP-5 column for **GC** analysis; the transfer line temperature was programmed from 150°C to 180°C; source temperature, 230°C; quadrupole temperature 150°C; carrier gas, helium adjusted to a linear velocity of 34 cm/s; ionization energy, 70 eV; scan range, 40 to 500 amu; 3.9 scans/s. A simple (1.0 µL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their **GC** retention indices, by comparing of their mass and those of standard components available in the laboratory, and through library search (Nist 05 and Wiley MS Data Library, 6th edn) [11,12].

Free radical scavenging activity: Free radical scavenging activity of essential oils were determined by using a stable free radical, namely **DPPH** (2,2-diphenyl-1-picrylhydrazyl), according to a slightly modified method of Blois, 1958. Indeed, since **DPPH** has an unpaired electron its delocalization, by reaction with an antioxidant substance gives a violet color to the **DPPH** solution. By donating a hydrogen radical, **DPPH** is stabilized producing a decrease in absorbance [13]. **DPPH** solution was prepared at the concentration of 6 x 10^{-2} mM of **DPPH** in ethanol. During assays, 1 mL of the essential oil (1000 µg/mL) was mixed with 3 mL **DPPH** solution. Simultaneously, a control (ascorbic acid) was prepared without essential oil. The mixture was incubated at room temperature for 30 min and further reading on a Spectronic Genesystm 10 Bio reader plate at 517 nm. The percentages of inhibition of the **DPPH** radical, as a function of the effect extracted fractions, were calculated using the following equation, where $\mathbf{A_{CO}}$: the absorbance of the control at t=0; $\mathbf{A_{AT}}$: the absorbance of the samples at t = 30 min.

The $\mathbf{IC_{50}}$ value was calculated based on the percentage of inhibition of each concentration, namely 500, 250, 125, 62.5, 31.25 and 15.62 µg/mL. The percentage of inhibition (**y**) from each concentration (**x**), the points (x and y) are plotted on the coordinate plane then the line equation **y = ax + b** is determined by calculation using linear regression where a and b are constants, **x** is the concentration sample (ppm), and **y** is the percentage of inhibition (%). Antioxidant activity is expressed by $\mathbf{IC_{50}}$, namely the sample's concentration that can reduce 50% of **DPPH** radicals [14, 15].

$$\text{Inhibition} = \frac{A_{co} - A_{at}}{A_{co}} * 100$$

RESULTS AND DISCUSSION

In this study the chemical composition of the essential oil of flowers and leaves of *M. mollis* collected in the Province of Chimborazo-Ecuador are reported. Hydrodistillation from the flowers of *M. mollis* produced a 0.45 mL yellow oil with a yield of 0.09%, while the leaves produced a 0.75 mL yielded 0.10% of oil. **GC-MS** analysis showed the presence of 12 components which were identified in the oil of the flowers and leaves, they represent 98.86 % and 99.99%, of the total oil, respectively. These compounds with their retention indices (**RI**) and relative percentage concentrations are listed in Table 1, according to the elution order on HP-5 column. The major components of the flowers oil were pulegone (45.19%), menthone (37.66%) and neomenthol (6.03%) and the major components of the leaves oil were pulegone (18.76%), menthone (46.58%), α-neomenthol (12.65%) and neomentil acetate (9.91%) and menthyl acetate (4.19%). The identified products may be divided into four different groups: hydrocarbons monoterpenes (1.58% flowers; 2.10% leaves), oxygenated monoterpenes (95.81% flowers; 96.71% leaves), sesquiterpenes (1.47% flowers; 1.18% leaves).

The essential oil of *M. mollis* obtained from plants collected at different places has been described. In the Venezuelan Andes the essential oils of *M. mollis* from leaves contain pulegone as their major component. However, the essential oil of *M. mollis* from seeds obtained in Ecuador contains menthol derivatives as their major

compounds. The analysis of the oil from plants raised at Carroll Co, Indiana, from seeds purchased in Quito, Ecuador, showed the presence of neomenthol (29.3%), menthol (20.6%), menthone (24.0%) and piperitone (9.0%); those collected at Zaruma (Province of El Oro, Ecuador) contained menthone (16.0%), carvacryl acetate (10.0%), pulegone (10.0%) and carvacrol (9.0%) [16-19].

TABLE 1.
Percentage composition of the essential oil from flowers and leaves of *Minthostachys mollis.*

Serial N°	Compounds	Flowers oil	Leaves oil	RI
1	α-terpinolene	1.58	2.10	1099
2	menthone	**37.66**	**46.58**	1159
3	neomenthol	**6.03**	**12.65**	1167
4	menthol	0.77	2.55	1174
5	pulegone	**45.19**	**18.76**	1246
6	piperitone	1.04	1.18	1261
7	neomenthyl acetate	2.48	9.91	1282
8	menthyl acetate	1.00	4.19	1300
9	piperitenone	0.78	0.29	1347
10	eugenol	0.86	0.60	1361
11	β-caryophyllene	0.71	0.59	1419
12	bicyclo-germacrene	0.76	0.59	1495
	Total Identified	**98.86%**	**99.99%**	

RI: Retention index this work.

All of them have been previously reported as characteristic components of different chemotypes of *M. mollis* from Argentina, these findings suggest large genetic diversity in *M. mollis* from Ecuador with regard to the main essential oil components which appears to be divided into two chemotypes: carvacryl acetate-carvacrol and predominantly pulegone-menthone [20, 21]. Many studies show that the chemical composition of essential oils varies noticeably according to numerous factors, including environmental geobotanical conditions, cultivation technique, plant age, harvest period, among others [22].

The antioxidant mechanism is mostly known as radical scavenging; this assay is based on measuring the reducing ability of antioxidants toward **DPPH**. This method is an *In Vitro* method that is often chosen for antioxidant activity because it is simple, easy, fast, sensitive, and requires a small sample. This method only requires **DPPH** without adding a substrate because free radicals are available directly to touch the substrate. The ability can be evaluated by electron spin resonance or by measuring the decrease of its absorbance [23]. The results can be observed by changing the solution from purple to yellow. Color changes indicate that **DPPH** has been reduced by the hydrogen or electron donation process of antioxidant compounds. The absorption decreases gradually as much as the concentration of antioxidant compounds. This method uses $\mathbf{IC_{50}}$ as a parameter to determine the concentration of antioxidant compounds that can inhibit 50% oxidation [23].

Table 2 showed that the $\mathbf{IC_{50}}$ of the essential oil of flowers and leaves of *M. mollis* were 32.73 µg/mL, and 15.40 µg/mL, respectively. These results indicated powerful antioxidant activity for both oils. The category as antioxidant based on **DPPH** assay divided into; powerful, strong, medium, and weak with the $\mathbf{IC_{50}}$ <50, 50-100, 100-250, 250-500 µg/mL, respectively [23].

TABLE 2.
Antioxidant activity of the essential oil of flowers and leaves of *M. mollis*.

Sample	Concentrations	Inhibition (%)	IC_{50} (µg/mL)
Ascorbic acid positive control	12.5	20.67	**35.20**
	25	37.09	
	50	69.02	
	75	96.36	
	100	97.08	
Essential oil of flowers *M. mollis*	15. 62	46.61	**32.73**
	31.25	50.36	
	62.5	54.97	
	125	58.78	
	250	63.82	
	500	71.88	
Essential oil of Leaves *M. mollis*	15.62	49.87	**15.40**
	31.25	51.57	
	62.5	53.93	
	125	55.69	
	250	59.39	
	500	65.75	

IC_{50}: Concentration that can reduce 50% of **DPPH** radicals

The antioxidant activity of essentials oils depends on the presence of bioactive components that can quench peroxyl radicals or inhibit the oxidation reaction of the organic materials. Their antioxidative effects have been attributed to the presence of various terpenes and phenolic compounds. Terpenes, especially from the aromatic such as linalool, eucalyptol, citral, citronellal, isomenthone, menthone, α-terpinene, β-terpinene, and α-terpinolene, have been widely applied as additives in food supplements for preventing oxidative stress. Additionally, antioxidant activities have also been associated with specific alcohols, ethers, aldehydes, and ketones [24-28]. It is important to highlight that some studies have reported that the radical scavenging activity of peppermint essential oils is associated with the presence of menthol and menthone [29-31].

Previous studies have reported that monoterpenes without a π bond, for example, menthol, do not exert free radical scavenging activity in the **DPPH•** assay. When one double bond appears in the molecule, the **DPPH•** scavenging ability increases, as, for example, with menthone. Compounds with two double bonds, as already explained, quickly terminate radical chain reaction and can be considered as potent free radical scavengers, for example, pulegone [32]. However, it must be considered that, natural essential oils are a mixture of different types of antioxidants or oxidizable terpenoids compounds. Depending on the composition and experimental conditions, synergistic or antagonistic interaction can occur and play an important role in the efficacy of the antioxidant action. Care should be taken before assuming that the antioxidant potential of essential oils is simply because of a specific terpene [33].

The essential oil of the leaves of *M. mollis* presented an **IC_{50}** of 15.40 µg/mL while the essential oil of the leaves was 32.73 µg/mL. It seems plausible that menthone and pulegone play a significant role in the antioxidant activity of the essential oils of *M. mollis*. It is observed that the antioxidant activity was higher in the oil of the leaves, this can be attributed to the synergistic effect of neomenthyl acetate and menthyl acetate, they are found in greater proportion in said oil and have double bonds in their structures (Fig 1).

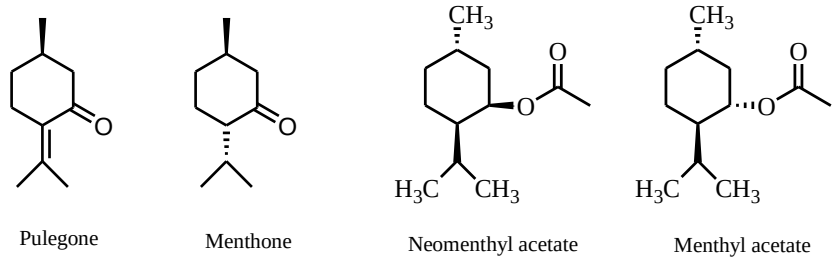

Fig. 1 Major compounds with double bonds of the essential oil of the of *M. mollis* leaves.

CONCLUSIONS

The chemical composition of essential oils of fresh flowers and leaves of *M. mollis* collected in the Province of Chimborazo were evaluated in the present investigation. The main components of the oil found in the flowers and leaves were pulegone (45.19%) and menthone (46.58%), respectively. The antioxidant test showed the essential oil of flowers and leaves of M. mollis had powerful antioxidant activity with **IC_{50}** values of 32.73 µg/mL and 15.40 µg/mL, respectively. The results of these studies provide scientific evidence of natural compounds with the potential to act as antioxidants again diseases in the human body.

BIBLIOGRAPHY REFERENCES

[1] Schmidt-Lebuhn AN. Revision of the genus *Minthostachys* (Labiatae). 1st edition Memoirs of the New York Botanical Garden. 2008; 1-77.

[2] Stonis JR, Diškus A, Fernández-Alonso JL, Remeikis A, Solis A. New leaf-mining *Nepticulidae* (Lepidoptera): potential pests of aromatic Lamiaceae plants from equatorial Andes. Zootaxa. 2021; 4926(3): 363-383. doi:10.11646/zootaxa.4926.3.3

[3] Schmidt-Lebuhn AN. Ethnobotany, biochemistry and pharmacology of *Minthostachys* (Lamiaceae). J. Ethnopharmacol. 2008; 118(3): 343-353. doi:10.1016/j.jep.2008.05.030

[4]] Lock O, Pérez E, Villar M, Flores D, Rojas R. Bioactive compounds from plants used in Peruvian traditional medicine. Nat. Prod. Commun. 2016; 11(3): 315-337.

[5] Cariddi L, Escobar F, Moser M, Panero A, Alaniz F, Zygadlo J, Sabini L, Maldonado A. Monoterpenes isolated from *Minthostachys verticillata* (Griseb.) Epling essential oil modulates immediate-type hypersensitivity responses *in vitro* and *in vivo*. Planta Med. 2011; 77(15): 1687-1694. doi: 10.1055/s-0030-1271090

[6] Montironi ID, Cariddi LN, Reinoso EB. Evaluation of the antimicrobial of *Minthostachys verticillata* essential oil and limonene against *Streptococcus uberis* strains isolated from bovine mastitis. Rev. Argent Microbiol. 2016; 48(3): 210-216. doi: 10.1016/j.ram.2016.04.005

[7] Mora FD, Araque M, Rojas LB, Ramirez R, Silva B, Usubillaga A. Chemical composition and *in vitro* antibacterial activity of the essential oil of *Minthostachys mollis* (Kunth) Griseb Vaught from the Venezuelan Andes. Nat. Prod. Commun. 2009; 4(7): 997–1000. doi: 10.1177/1934578X0900400726

[8] Rojas-Armas JP, Arroyo-Acevedo JL, Ortiz-Sánchez JM, Palomino-Pacheco M, Hilario-Vargas HJ, Herrera-Calderón O, Hilario-Vargas J. Potential toxicity of the essential oil from *Minthostachys mollis*: A medicinal plant commonly used in the traditional Andean medicine in Peru. J. Toxicol. 2019; 19(1): 1-9. doi:10.1155/2019/1987935

[9] Gleiser R, Bonino M, Zygadlo J. Bioactividad de aceites esenciales de *Minthostachys mollis* contra mosquitos. Bol. Latinoam. Caribe Plant. Med. Aromaticas. 2007; 6(6): 350-351.

[10] Quezada-Moreno WF, Quezada-Torres WD, Travez A, Arias G, Cevallos E, Zambrano Z, Brito H, Salazar K. Essential oil of *Minthostachys mollis*: extraction and chemical composition of fresh and stored samples. J. Medicinal Aromat. Plants. 2019; 5: 59-71. doi:0.48347/IMIST.PRSM/ajmap-v5i1.15684

[11] Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20 M. phases. J. Chromatogr. A. 1990; 503: 1-24. doi:10.1016/S0021-9673(01)81487-4

[12] Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corp., Carol Stream, Illinois. 2007; 1-804. doi:10.1016/j.jasms.2005.07.008

[13] Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 2004; 26(2): 211-219.

[14] Akar Z, Küçük M, Doğan H. A new colorimetric DPPH• scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. J Enzyme Inhib Med Chem. 2017; 32(1): 640-647. doi:10.1080/14756366.2017.1284068.

[15] Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM, García-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2007; 71(1): 230-235 .doi:10.1016/j.talanta.2006.03.050

[16] Calle JC, Espinosa AM, Nuñez CP, Bautista E, Pinzón R. Actividad insecticida del aceite esencial de *Mintostachys mollis* (HBK) Griseb y sus componentes. Rev. Colomb. Cienc. Quim. Farm. 2004; 33(2): 137-144.

[17] Rojas LB, Usubillaga AN. Essential oil of *Minthostachys mollis* Griseb bach from Venezuela. J. Essent. Oil Res. 1995; 7(2): 211–213. doi:10.1080/10412905.1995.9698503

[18] Alkire BH, Tucker AO, Maciarello MJ. Tipo, *Minthostachys mollis* (Lamiaceae): an Ecuatorian mint. Econ Bot. 1994; 48: 60-64. doi:10.1007/BF02901380

[19] Malagón O, Vila R, Iglesias J, Zaragoza T, Cañigueral S. Composition of the essential oils of four medicinal plants from Ecuador. Flavour Fragr J. 2003; 18(6): 527–531. doi:10.1002/ffj.1262

[20] Zygadlo UA, Maestri DM, Lamarque A, Guzman CA, Velasco-Negueruela A, Pérez-Alonso M, García-Vallejos M, Grosso NR. Essential oil variability of *Minthostachys verticillata*. Biochem. Syst. Ecol. 1996; 24(4): 319-323. doi: 10.1016/0305-1978%2896%2900030-0

[21] Baren C, Di Leo Lira D, Elechosa M, Molina N, Juarez M, Martínez A, Perelman S, Bandoni A. New insights into the chemical biodiversity of *Minthostachys mollis* in Argentina. Biochem. Syst. Ecol. 2014; 57: 374-383. doi:10.1016/j.bse.2014.09.004

[22] Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJ. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flav Fragr J. 2008; 23(4); 213-226. doi:10.1002/ffj.1875

[23] Kato S, Aoshima H, Saitoh Y, Miwa N. Highly hydroxylated or γ-cyclodextrin-bicapped water-soluble derivative of fullerene: The antioxidant ability assessed by electron spin resonance method and β-carotene bleaching assay. Bioorg. Med. Chem. Lett. 2009; 19(18): 5293-5296. doi:10.1016/j.bmcl.2009.07.149.

[24] Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential oils' chemical characterization and investigation of some biological activities: A Critical Review. Medicines. 2016; 3(4): 25. doi:10.3390/medicines3040025

[25] Olmedo R, Ribotta P, Grosso NR. Antioxidant activity of essential oils extracted from *Aloysia triphylla* and *Minthostachys mollis* that improve the oxidative stability of sunflower oil under accelerated storage conditions. Eur. J. Lipid Sci. Technol. 2018; 120(8): 1800374. doi:10.1002/ejlt.201700374

[26] Torres-Martínez R, García-Rodríguez YM, Ríos-Chávez P, Saavedra-Molina A, López-Meza JE, Ochoa-Zarzosa A, Garciglia RS. Antioxidant activity of theeEssential oil and its major terpenes of *Satureja macrostema* (Moc. and Sessé ex Benth.) Briq. Pharmacogn Mag. 2018; 13(4): S875–S880. doi:10.4103/pm.pm_316_17

[27] Chircov C, Miclea II, Grumezescu V, Grumezescu AM. Essential oils for bone repair and regeneration-mechanisms and applications. Materials. 2021; 14(8): 1867-1887. doi:10.3390/ma14081867

[28] Basavegowda N, Baek KH. Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications. Biomolecules. 2021; 11(9): 1267-1285. doi:10.3390/biom11091267

[29] Wu Z, Tan B, Liu Y, Dunn J, Martorell Guerola P, Tortajada M, Cao Z, Ji P. Chemical composition and antioxidant properties of essential oils from peppermint, native Spearmint and Scotch Spearmint. Molecules. 2019; 24(15): 2825. doi:10.3390/molecules24152825

[30] Sun Z, Wang H, Wang J, Zhou L, Yang P. Chemical composition and anti-inflammatory, cytotoxic and antioxidant activities of essential oil from leaves of *Mentha piperita* grown in China. PLoS ONE. 2014; 9(12): e114767. doi:10.1371/journal.pone.0114767

[31] Do Nascimento LD, Barbosa de Moraes AA, Santana da Costa K, Pereira-Galúcio J M, Taube P S, Leal C M, Neves J, Aguiar-Andrade H, Guerreiro de Faria L. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: New findings and potential applications. Biomolecules. 2020; 10(7): 988-1022. doi:10.3390/biom10070988

[32] Wojtunik K, Ciesla L, Waksmundzka M. Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2,2-Diphenyl-1-picrylhydrazyl method. J Agric Food Chem. 2014; 62(1): 9088−9094. doi: 10.1021/jf502857s

[33] Riachia L, De Maria C. Peppermint antioxidants revisited. Food Chem. 2015: 176(1): 72-81. doi: 10.1016/j.foodchem.2014.12.028

Toaquiza-Aguagallo Cecilia, Orcid ID: 0000-0002-5068-6691

Cando-Brito Verónica, Orcid ID: 0000-0001-9290-8523

†Rojas-Fermín Luis, Orcid ID: 0000-0003-4508-1927

Pérez-Colmenares Alida, Orcid ID: 0000-0001-8910-4663

Aparicio-Zambrano Rosa, Orcid ID: 0000-0002-5020-0954

Obregón-Díaz Ysbelia, Orcid ID: 0000-0001-6152-6696