Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 154-170
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Characterization of the Biological Potential of the Essential Oils from Five Species of Medicinal Plants

Danubia Aparecida Carvalho Selvati Rezende[1], Rafaela Vieira Souza[1], Maisa Lamounier Magalhães[2], Alex Rodrigues Silva Caetano[1], Marcos Schleiden Sousa Carvalho[3], Ellen Cristina de Souza[2], Luiz Gustavo de Lima Guimarães[4], David Lee Nelson[5], Luís Roberto Batista[2], Maria das Graças Cardoso[1*]

[1]Departamento de Química, Universidade Federal de Lavras, Lavras, Brasil
[2]Departamento de Ciência dos Alimentos, Universidade Federal de Lavras, Lavras, MG, Brasil
[3]Departamento de Agricultura, Universidade Federal de Lavras, Lavras, MG, Brasil
[4]Universidade Federal de São João del-Rei, São João del-Rei, Brasil
[5]Universidade Federal do Vale do Jequitinhonha e Mucuri, Diamantina, MG, Brasil
Email: *mcardoso@dqi.ufla.br

How to cite this paper: Rezende, D.A.C.S., Souza, R.V., Magalhães, M.L., Caetano, A.R.S., Carvalho, M.S.S., de Souza, E.C., Guimarães, L.G. de L., Nelson, D.L., Batista, L.R. and Cardoso, M. das G. (2017) Characterization of the Biological Potential of the Essential Oils from Five Species of Medicinal Plants. *American Journal of Plant Sciences*, **8**, 154-170.
http://dx.doi.org/10.4236/ajps.2017.82012

Received: November 24, 2016
Accepted: January 17, 2017
Published: January 20, 2017

Abstract

Essential oils from *Mentha piperita, Cymbopogon citratus, Rosmarinus officinalis, Peumus boldus* and *Foeniculum vulgare* were extracted by hydrodistillation, chemically characterized and quantified by GC/MS and GC/FID. The principal componentes in the essential oil of *M. piperita* (carvone and limonene), *C. citratus* (geranial, neral and myrcene), *R. officinalis* (1,8-cineole, camphor and *α*-pinene), *P. boldu*s (*α*-terpinyl formate, *p*-cymene and 1,8-cineole) and *F. vulgare* (methyl chavicol, limonene and fenchone) were identified. The oils were tested for antioxidant activity employing the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical-capture method, the oxidation of the *β*-carotene/linoleic acid system, and the ABTS radical-capture method. The essential oils were not effective. The antioxidant activities of the oils were determined by the hydroxyl method, and the highest activity (62.80%) was observed with the essential oil from *M. piperit*a. Activities of 1.54 and 1.82 μg μg^{-1}, respectively, were observed for the essential oils from *C. citratus* and *P. Boldus* using the phosphomolybdenum method. No activity was observed with respect to reducing power. The essential oil from *C. citratus* was effective in inhibiting the growth of *L. monocytogenes* (15.63 $mg \cdot L^{-1}$), *S. choleraesuis* (15.63 $mg \cdot L^{-1}$) and *E. coli* (31.25 $mg \cdot L^{-1}$), and the essential oil from *P. boldus* inhibited the growth of *S. aureus* (62.5 $mg \cdot L^{-1}$). No essential oil inhibited the growth of *P. aeruginosa*. A low antioxidant activity and a promising antibacterial activity were observed for these essential oils.

Keywords

Natural Products, Antioxidant, Antibacterial Activity

1. Introduction

Since ancient times, plants have been used to treat, prevent and cure diseases. However, the use of plants with medicinal potential has increased considerably and has stimulated studies that seek the characterization, identification and isolation of new natural products with medicinal properties. Among these natural products, the essential oils, regarded as flavorings or essences, have many applications because of their medicinal properties and their importance in the cosmetics and food industries. Essential oils are complex mixtures of volatile substances extracted from the plant by steam distillation or hydrodistillation. They are colorless or light yellow and are generally odoriferous. They have a high biological potential; they may have antioxidant, antiseptic, antimicrobial, antifungal, anti-inflammatory, or repellent activities, among others [1].

Antioxidants are substances that prevent or retard the rate of oxidation through one or more mechanisms that involve inhibiting or reducing the effects triggered by free radicals and oxidizing compounds on oxidizable substrates. Many compounds produced by plants have been the object of research regarding their antioxidant potentials, and the results have been promising. Among these substances, the essential oils stand out because many of their components can replace or be associated with synthetic substances. The acceptance of these products by consumers results in expansion of the use of essential oils of various plants as raw materials in food, pharmaceutical, and cosmetic industries [2].

Pathogenic bacteria have been the cause of the principal problems arising from the proliferation of resistant micro-organisms, especially with respect to the contamination of various foods. Food poisoning outbreaks are increasingly common, so there is a need to find compounds that are able to control bacterial strains resistant to antibiotics so as to reduce the contamination and deterioration of food-products [3]. This study sought to chemically characterize the essential oils from the following medicinal plants: *M. piperita*, *C. citratus*, *R. officinalis, P. boldus* and *F. vulgare* and to evaluate their antioxidant and antibacterial activities.

2. Material and Methods

2.1. Extraction of Essential Oils

Approximately 1.0 kg of dry material (leaves) from each plant (*Mentha piperita*, *Cymbopogon citratus*, *Rosmarinus officinalis*, *Peumus boldus* and *Foeniculum vulgare*), acquired in the Municipal Market of Belo Horizonte, Minas Gerais, Brazil, was used. The essential oils of selected medicinal plants were extracted in the Organic Chemistry-Essential Oils Laboratory of the Chemistry Department of the Federal University of Lavras. The material was extracted by hydrodistilla-

tion using a modified Clevenger apparatus coupled to a 5 L flask [4]. A 300 g quantity of each dried plant material was subjected to the hydrodistillation process for two hours. After extraction, the essential oil was separated from the hidrolact by centrifugation using a benchtop centrifuge with a horizontal crosspiece (FANEM Baby®I Model 206 BL) at 965*g* at room temperature for 5 minutes. The essential oil was transferred to an amber bottle with the aid of a Pasteur pipette and stored at 5˚C.

2.2. Identification and Quantification of Essential Oils

The identification of the constituents of the essential oils was performed by gas chromatography coupled to a mass spectrometer using a Shimadzu QP model 5050A GC/MS equipped with a J&W Scientific fused-silica capillary column (5% phenyl 95% dimethylpolysiloxane; 30 m × 0.25 mm id, 0.25 μm of film) using He as the carrier gas with a flow rate of 1.2 mL min^{-1}. The column temperature was maintained at 50˚C for 2 min, increased at 4˚C min^{-1} to 200˚C, increased at 15˚C min^{-1} to 300˚C, and maintained at this temperature for 15 min. The temperatures of the injector and interphase were maintained at 250˚C and 280˚C, respectively. A 0.5 μL sample of the oil in ethyl acetate was injected. The configuration of the mass spectrometer (MS) included an ion capture detector operating in electron impact mode and an impact energy of 70 eV. The scan speed was 1000; the scan interval was 0.50, and fragments were detected in the range of 40 to 500 Da. The retention indices of the constituents were compared with those of the literature [5]. The retention indices were calculated using the equation of Van Den Dool and Kratz [6] relative to a homologous series of n-alkanes (C_9-C_{18}). Two libraries, NIST107 and NIST21, were used for comparison of the mass spectra.

The quantitative analyses were conducted by gas chromatography (Shimadzu GC-17A) using a flame ionization detector (FID) and a ZB-5MS fused-silica capillary column (5% phenyl-95% dimethylpolysiloxane; 30 m × 0.25 mm id × 0.25 μm film). The carrier gas was He with a flow rate of 1.2 mL·min^{-1}. The analytical conditions were the same as those used for the GC-MS analysis, and the concentration of each component was obtained by normalization of areas (%).

2.3. Antibacterial Activity of Essential Oils

To assess the antibacterial activities, the following micro-organisms were used: *Listeria monocytogens* ATCC19117, *Salmonella* Choleraesuis ATCC6539, *Pseudomonas aeruginosa* ATCC15442, *Escherichia coli* ATCC11229 and *Staphylococcus aureus* ATCC13565. Initially, cultures were activated in broth (BHI) with incubation at 37˚C for 24 hours. After activation, the cultures were transferred to a tube with 5 mL of tryptic soy broth (TSB) and incubated at 37˚C to achieve the turbidity of a McFarland 0.5 standard solution, resulting in a concentration of 108 colony forming units (CFU) per milliliter. The turbidity readings were performed using a spectrophotometer (Varian, Cary 50 probe) at a wavelength of 625 nm [7].

The cultures were poured into petri plates containing a thin layer of fresh

Mueller-Hinton Agar (20 mL), and sterile glass beads were placed on this layer. A second layer of agar containing the inoculated microorganisms was poured over the first layer and the glass beads. After solidification, the glass beads were removed, and 10 µL of DMSO solution of the essential oils at concentrations of 15.62, 31.25, 62.5, 125, 250, and 500 $\mu g \cdot mL^{-1}$ was placed in each well. The plates were incubated in BOD at 37˚C for 24 hours. The diameters of the inhibition halos formed were measured, and the minimum inhibitory concentration (MIC), the lowest concentration that inhibited the growth of the microorganism, was calculated. Chloramphenicol (standard antibiotic) was used as a positive control, and DMSO as the negative control [8]. The micro-organisms *S. choleraesuis, P. aeruginosa, E. coli* and *S. aureus* were transferred to plates containing Mueller-Hinton Agar. Casoy Agar (TSA) supplemented with yeast extract [9] was used for *L. monocytogens*.

2.4. Antioxidant Activities of the Essential Oils

The antioxidant activities of the essential oils were determined using the methods that evaluate the capacity for sequestration of the DPPH radical, inhibition of the oxidation of the β-carotene/linoleic acid system, stabilization of the ABTS radical, capture of the hydroxyl radical, the reducing power and the reduction of molybdenum.

2.5. Sequestering of the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical

A solution of DPPH in ethanol (stock solution) was prepared at a concentration of 40 $\mu g\ mL^{-1}$. The stock solution (2700 µL) was added to test tubes followed by the addition of 300 µL of the essential oil diluted in ethanol at concentrations of 25, 50, 100, 150, 200, 250 and 500 $\mu g\ mL^{-1}$. The control contained all the reagents except the essential oil. After incubation for 60 minutes in the absence of light, readings were performed in a spectrophotometer at a wavelength of 515 nm. Comparison tests were conducted with synthetic BHT (butylated hydroxytoluene) at the same concentration [10].

2.6. Oxidation of the β-Carotene/Linoleic Acid System

To evaluate the inhibition of oxidation of the β-carotene/linoleic acid system, 60 mg of linoleic acid, 600 mg of Tween 20, 6 mg of β-carotene and 30 mL of chloroform were added to a round-bottom flask, and the mixture was evaporated using a rotary evaporator at 50˚C. After complete removal of the chloroform, 150 mL of distilled water saturated with oxygen was added. To 2700 µL of this solution in test tubes, was added 300 µL of the essential oil diluted in ethanol at concentrations of 25, 50, 100, 150, 200, 250 and 500 $\mu g\ mL^{-1}$. The control contained only ethanol. The absorbance was immediately measured in a spectrophotometer at 470 nm. The tubes were incubated at 50˚C for 60 minutes to effect the oxidation, and a second reading was taken. The blank was prepared in the same manner as the emulsion with the addition of all the reagents except β-carotene. For comparison, the BHT standard was tested using the same concentrations [11].

2.7. Stabilization of the ABTS Radical

The ABTS$^{\bullet}$ radical was formed by reaction of ABTS with 2.45 mM potassium persulphate at 25˚C for 12 - 16 h in the absence of light. After the formation of the radical, it was diluted in ethanol to obtain an absorbance between 0.7 to 0.72 at 734 nm (Shimadzu UV-1601PC). To 1900 µL of the ABTS$^{\bullet}$ in a test tube, was added 100 µL of the samples at concentrations of 25, 50, 100, 150, 200, 250 and 500 µg·mL^{-1}. The synthetic antioxidant BHT was employed at the same concentrations as a positive control [12].

2.8. Neutralization of the Hydroxyl Radical

The method described by Boulanouar *et al.* [13] was used to evaluate the antioxidant activity through the capture of the hydroxyl radical (OH$^{\bullet}$). To a test tube, 100 µL of the sample diluted in water at concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL^{-1}, 100 µL of the mixture of $FeSO_4$/EDTA, 100 µL of deoxyribose, 700 µL of 0.1 M phosphate buffer (pH = 7.4) and 100 µL of H_2O_2 were added. The tubes were incubated at 50˚C for 60 min, and 500 µL each of trichloroacetic acid (TCA) and thiobarbituric acid were added. The tubes were boiled for 10 min and cooled; the absorbance was measured at 532 nm in a Shimadzu UV-1601PC spectrophotometer. Mannitol was employed as the standard.

2.9. Reducing Power

Fifty-microliter aliquots of the essential oils at concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL^{-1} were added to 500 µL of 200 mM phosphate buffer, pH 6, and 500 µL of 1% potassium hexacyanoferrate III. The mixture was stirred and incubated at 50˚C for 20 minutes. After the addition of 500 µL of 10% TCA, 1500 µL of distilled water and 300 µL of 0.1% $FeCl_3$, the absorbance was measured at 700 nm, and a plot of absorbance versus sample concentration was constructed. Ascorbic acid was used as the standard [14].

2.10. Reduction of Molybdenum

To test tubes were added 50 µL of solutions of the essential oils at different concentrations (25, 50, 100, 150, 200, 250 and 500 µg mL^{-1} in ethanol), 2.000 mL of ammonium phosphomolibdate (10% sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were stirred and incubated at 95˚C for 60 minutes. After cooling, the absorbance was measured at 695 nm. The synthetic standard was ascorbic acid [15].

2.11. Statistical Analysis

The data resulting from the antioxidant studies were submitted to analysis of variance using a completely randomized design in a 5 × 7 factorial scheme (essential oils × concentrations), and the means were compared by the Scott-Knott test at 5% probability. The statistical program used was SISVAR [16].

3. Results and Discussion

The chemical compositions of the essential oils from *Mentha piperita*, *Cymbopogon citratus*, *Rosmarinus officinalis*, *Peumus boldus* and *Foelniculum vulgare* are shown in Table 1 where the principal components present in each essential oil are emphasized.

Table 1. Compositions of the essential oils from *Mentha piperita*, *Cymbopogon citratus*, *Rosmarinus officinalis*, *Peumus boldus* and *Fóleo essencialniculum vulgare*.

RI	Constituent	Percent of constituent				
		M. piperita	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
927	*α*-pinene	-	-	.	0.92	-
942	camphene	-	-	2.55	-	-
963	sabinene	-	-	-	0.59	-
970	*β*-pinene	-	-	1.41	0.34	-
980	myrcene	0.55	**8.46**	-	-	-
1015	*p*-cymene	-	-	-	**15.45**	-
1022	limonene	**10.97**	-	-	-	**6.15**
1028	1.8-cineol	-	-	**62.26**	**10.59**	-
1082	fenchone	-	-	-	0.17	3.8
1089	linalool	-	2.46	-	-	-
1143	camphor	-	-	**17.34**	-	-
1155	pinocarvone	-	-	-	0.45	-
1162	isoborneol	-	-	3.77	-	-
1169	terpinen-4-ol	-	-	-	2.03	-
1185	*α*-terpineol	-	-	2.77	-	-
1200	methylchavicol	-	-	-	-	**89.48**
1231	ascaridol	-	-	-	2.73	-
1236	neral	-	**35.43**	-	-	-
1245	geraniol	-	2.16	-	-	-
1246	carvone	**84.34**	-	-	-	-
1247	cis-epoxy-piperitone	-	-	-	0.43	-
1266	geranial	-	**47.74**	-	-	-
1281	2-undecanone	-	1.68	-	-	-
1305	*α*-terpinyl formate	-	-	-	**61.99**	-
1372	geranyl acetate	-	0.20	-	-	-
1383	*β*-bourbunene	0.46	-	-	-	-
1419	E-cariophylene	0.75	-	-	-	-
1428	*β*-copaene	0.09	-	-	-	-
1454	*α*-humulene	0.11	-	-	-	-
1462	cis-muurola-4(14).5-diene	0.22	-	-	-	-
1607	*β*-oplopenone	-	-	-	0.14	-
1616	1,10-di-epi-cubenol	0.17	-	-	-	-
1655	*α*-cadinol	0.18	-	-	-	-
	Total	97.84	98.13	99.17	95.83	99,46

RI = Retention index.

Regarding the essential oil from *M. piperita*, the princpal constituents were carvone (84.34%) and limonene (10.97%). Recent studies verified the presence of 18 compounds in the essential oil from *M. piperita*, and the principal components were pulegone (45.00%) followed by iso-menthol (12.80%), piperitanona (9.10%), menthone (8.00%), piperitone (7.40%) and piperitanone oxide (6.90%) [17]. Their data are different from those found in this work. This difference can be explained by edafoclimatic differences such as the locations and hours of collection, soil, nutrients, among others, because the plants were collected in different locations [18].

The principal constituents of the essential oil from *C. citratus* were geranial (47.74%), neral (35.43%) and myrcene (8.46%). The principal components found in this study are consistent with those obtained by Miranda *et al.* [19] who studied the essential oil from *C. citratus* specimens collected in Lavras, MG, Brazil. However, those authors found different proportions of geranial (41.61%), neral (29.78%) and myrcene (2.07%).

The principal constituents in the essential oil from *R. officinalis* were 1,8-cineole (62.26%), camphor (17.34%) and *α*-pinene (9.07%). Lemos *et al.* [20] found the same principal components in the essential oil from *R. officinalis*, but in very different quantities. The authors found a higher percentage of camphor (24.38%), followed by 1,8-cineole (19.74%) and *α*-pinene (15.18%).

The principal components of the essential oil from *P. boldus* were *α*-terpinyl formate (61.99%), *p*-cymene (15.45%), 1,8-cineole (10.59%), ascaridol (2.73%), and terpinen-4-ol (2.03%). Vogel *et al.* [21] found ascaridol to be the main component of the essential oil from this species, whereas Villa *et al.* [22] obtained an essential oil containing limonene (17.0%), *p*-cymene (13.6%) and 1,8-cineole (11.8%), and only 1% of the terpenoids corresponded to ascaridol. The authors attributed this alteration to different varieties of *P. Boldus*.

According to the data presented in **Table 1**, the principal component of the essential oil from *F. vulgare* was methyl chavicol, also known as estragole (89.48%), followed by limonene (6.15%) and fenchone (3.80%). In recent studies by Evrendilek [17] of the essential oil from *F. vulgare* extracted from fennel seeds acquired in Hatay markets (Turkey), seven compounds were found; the principal component was *trans*-anethole (80.4%). These results are different from those found in the present work where no *trans*-anethole was found.

According to the observations of Gobbo-Neto and Lopes [18], the differences in the chemical compositions of the essential oils from medicinal plants of the same species can be due to several factors such as location and time of collection, type of soil and nutrients because the secondary metabolites in the plant can vary with the season in which it is collected. The age of the plant, seasonality, temperature, and other factors also influence virtually all the classes of secondary metabolites, including the essential oils. The essential oils contained a larger concentration of monoterpenes (oxygenated or not) and sesquiterpenes (oxygenated or not), whereas the essential oil from *F. vulgare* was the only one that contained a phenylpropanoid.

3.1. Antibacterial Activity of Essential Oils

The minimum inhibitory concentrations (MICs) of the essential oils tested against Gram-negative and Gram-positive bacteria are described in Table 2. The growth of *S. choleraesuis* and *L. monocytogenes* was inhibited by the essential oil from *C. citratus* at a minimum concentration of 15.63 μg·mL^{-1}. *E. coli* was more sensitive to the essential oils from *C. citraus* and *P. boldus*, both presenting a MIC of 31.25 μg·mL^{-1}. *P. aeruginosa* was not sensitive to any of the essential oils tested. *S. aureus* is a micro-organism that was sensitive only to the essential oil from *P. boldus* (62.5 μg·mL^{-1}). Antibacterial activity against Gram- positive and Gram-negative bacteria was observed for the essential oils from *M. piperita*, *C. citratus* and *P. boldus*. These results differ from those of most studies in which Gram-positive bacteria are reported to be more sensitive to essential oils because they have no outer membrane to act as a protective barrier against macromolecules and hydrophobic compounds. Because of this morphology, Gram-positive bacteria are relatively less resistant to antibiotics and other hydrophobic drugs, as well as essential oils [23].

Valeriano *et al.* [24], analyzing the antibacterial activity of the essential oil from *M. piperita* against the same bacterial strains evaluated in this study, observed that this species had a higher activity against *E. coli* and a low activity against *L. monocytogenes*. According to the authors, menthol, neoisomenthol, menthone, methyl acetate and iso-menthone were the principal components, whereas in the present work, similar activities against both *E. coli* and *L. monocytogenes* were observed for the essential oil from this species, and the principal constituents found were carvone and limonene.

Studies report that menthol is active against various micro-organisms, including *P. aeruginosa* [24]. Subsequently, work performed by Valeriano *et al.* [24] also showed that menthol is not the only agent responsible for the antimicrobial properties of *M. piperita*. The observations made by Iscan *et al.* [25] are

Table 2. Antibacterial activity of essential oils from *Mentha piperita*, *Cymbopogon citratus*, *Rosmarinus officinalis*, *Peumus boldus* and *Foeniculum vulgare*.

Bacteria	Gram	DMSO	CF	MIC (μg·mL^{-1})				
				M. piperita	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
S. choleraesuis ATCC6539	-	NI	100	**62.50**	**15.63**	125	125	125
E. coli ATCC11229	-	NI	100	**62.50**	**31.25**	125	**31.25**	**62.5**
P. aeruginosa ATCC 15442	-	NI	100	NI	NI	NI	NI	NI
S. aureus ATCC13565	+	NI	100	250	125	125	**62.5**	NI
L. monocytogenes ATCC19117	+	NI	100	**62.50**	**15.63**	500	250	125

NI: no inhibition, CF: chloramphenicol (C+).

not consistent with the findings of the present study because we observed no activity for the essential oil of this species against *P. aeruginosa*.

According to Silva *et al.* [26], the essential oil from *C. citratus* inhibited the growth of *S. aureus* (Gram-positive), and the MIC was 7.81 μg·mL^{-1}. The growth of *P. aeruginosa* and *E. coli* (Gram negative) was also inhibited with an average MIC of 15.62 μg·mL^{-1}. The results of the present work partially corroborate their results [26]; the MIC for the essential oil from *C. citratus* in this work was 15.63 μg·mL^{-1} for the inhibition of the growth of *L. monocytogenes* and *E. coli.*

3.2. Antioxidant Activity of Essential Oils

The percentages of antioxidant activities of the essential oils and BHT determined by the DPPH radical sequestration method are presented in Table 3. A low antioxidant activity was observed for the essential oils. A higher antioxidant activity was observed for the BHT standard than for the essential oils. The activities of the essential oils from *M. piperita*, *C. citratus* and *R. officinalis* did not differ significantly in any of the concentrations tested. As for the essential oil from *P. boldus*, there was a significant difference at the highest concentration (500 μg·mL^{-1}); the same effect was observed with the essential oil from *F. vulgare*.

The low antioxidant activity of the essential oils is related to their composition because no compound that could easily donate a hydrogen atom to stabilize the DPPH radical, nor any compound having a conjugated double bond, where stabilization could also occur, was found. Yadegarinia *et al.* [27] conducted research on the antioxidant activity of the essential oil from *M. piperita* using the DPPH method and observed an activity of 69%. The principal components of the essential oil were *α*-terpinene (19.7%), piperitenona oxide (19.3%), *trans*-carveol (14.5%) and isomenthone (10.3%). Their data do not match those found in this study.

Table 3. Percentage of antioxidant activity of the essential oils and the BHT standard measured by the sequestration of DPPH radicals.

Concentration (μg·mL^{-1})	Antioxidant activity (%)					
	BHT	*M. piperita*	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
25	13.90gA	0.39aB	0.00aB	0.11aB	0.25cB	0.05bB
50	22.80fA	0.54aB	0.20aB	0.01aB	0.18cB	0.00bB
100	38.50eA	0.66aB	0.20aB	0.00aB	0.00cB	0.00bB
150	44.60dA	0.51aB	0.19aB	0.01aB	1.25bB	0.69aB
200	52.50cA	0.52aD	0.34aD	0.06aD	2.32aB	1.36aC
250	59.70bA	0.38aC	0.15aC	0.00aC	1.91aB	1.12aB
500	72.70aA	1.32aC	0.56aD	0.43aD	2.40aB	1.34aC

Means followed by the same lower case letter in the columns and the same upper case letter in the row do not differ significantly by the Scott-Knott test ($p \leq 0.05$).

Guimarães *et al.* [28] studied the antioxidant activity of the essential oil from *C. citratus* and its main constituent, citral, via the capture of the DPPH radical and observed a low antioxidant activity for both. According to the authors, this fact can be justified by the fact that it is difficult for the constituents to donate a hydrogen atom to stabilize the DPPH radical. These results are consistent with those found in this work.

Teixeira *et al.* [29] assessed the antioxidant activity of the essential oil from *R. officinalis* using the DPPH-sequestration method and observed no significant potential for the samples when compared to the standard, because the principal compounds identified by the authors were camphor (35.50%), 1,8-cineole (18.20%), and bornyl acetate (13.40%). Camphor (17.34%) and 1,8-cineole (62.26%) were also found in this work, but in different concentrations. According to the authors, the low activity of the essential oil from *R. officinalis* is due to the constituents present in the oil, which did not possess structures favorable for donating a hydrogen atom or an electron to stabilize the DPPH radical.

Among the constituents of the essential oil from *P. boldus,* terpinen-4-ol has a hydroxyl group (-OH) that could donate a hydrogen atom, but there would be no stabilization of the resulting alkoxy radical by resonance. Therefore, its antioxidant potential by the DPPH method is low.

Roby *et al.* [30] observed a high antioxidant activity for *F. vulgare* seeds by the DPPH method. The authors found *trans*-anethole (56.40%), fenchone (8.26%), and estragole (5.20%) in the essential oil from this plant. In the present work, *trans*-anethole (a phenylpropanoid) was not identified in the essential oil from this species, but another phenylpropanoid, estragole, was identified. *Trans*-anethole is a compound that can donate an electron, and the resulting radical can be stabilized by resonance because it has a conjugated double bond. This feature is not present in the structure of estragole, and this fact may explain the low activity observed in this work because it was found to be the principal compound.

The results obtained for evaluation of the antioxidant activity of the essential oils and BHT using the *β*-carotene/linoleic acid method are described in **Table 4**. The highest antioxidant activity was observed for the essential oil from *C. citratus* (5.81%) at a concentration of 500 μg L^{-1}, followed by *F. vulgare*, *P. boldus* and *M. piperita*, which differed statistically from the control but possessed a low activity.

A higher antioxidant activity at the concentration of 150 μg L^{-1} was observed for the essential oils from *M. piperita* and *R. officinalis,* whereas a higher antioxidant activity in the two highest concentrations was observed for the essential oil from *F. vulgare.* The result was statistically different from those of the other essential oils. An increase in antioxidant activity with concentration was found for the essential oil from *P. boldus*, and the highest activity was observed at a concentration of 500 μg·mL^{-1} (3.37%)

Yadegarinia *et al.* [27] analyzed the antioxidant activity of the essential oil from *M. piperita L.* They found a 50.17% inhibition of oxidation using the *β*-carotene/linoleic acid method. These data do not corroborate those found in the

Table 4. Percentages of antioxidant activity of essential oils and the BHT standard by the β-carotene/linoleic acid method.

Concentration (μg·mL^{-1})	Antioxidant Activity (%)					
	BHT	*M. piperita*	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
25	63.40eA	0.28bC	1.81cB	0.00bC	0.28cC	0.16bC
50	74.00dA	0.76bB	1.72cB	0.00bB	0.75cB	0.88bB
100	81.30cA	1.09bC	3.83bB	0.40bC	1.36cC	0.09bC
150	81.90cA	2.12aC	4.56bB	0.99aC	1.67bC	0.92bC
200	85.50bA	2.26aC	4.12bB	1.26aC	1.84bC	1.25bC
250	86.60bA	2.03aC	4.16bB	1.92aC	2.08bC	2.95aC
500	91.00aA	3.11aC	5.81aB	1.64aD	3.37aC	3.96aC

Means followed by the same lower case letter in the columns and the same upper case letter in the row do not differ significantly by the Scott-Knott test ($p \leq 0.05$).

present work because the principal constituents identified by those authors (α-terpinene, piperitone oxide, *trans*-carveol, isomenthone and β-caryophyllene) were different from those found in the present study (carvone and limonene).

Guimarães *et al.* [28] determined the antioxidant activity of the essential oil from *C. citratus* and its principal constituent (citral) employing the emulsified β-carotene/linoleic acid system and noted that these substances exhibited an antioxidant activity of 46.45% and 38.00%, respectively, at an essential oil or citral concentration of 100 μg·L^{-1}. Their data did not corroborate those found in this study. The authors compared the antioxidant activity of the essential oil and citral and observed a higher activity for the essential oil than for citral. This fact shows that the principal constituent is not always the agent that is responsible for the activity, but rather all the components might act in synergy.

Gachkar *et al.* [31] evaluated the antioxidant activity of the essential oil from *R. officinalis* and found that the principal constituents were piperitone (23.70%), α-pinene (14.90%), linalool (14.90%) and 1.8-cineol (7.43%). They observed approximately 60% inhibition of oxidation and concluded that this oil can be used in food preservation. Their results are different from those found in this work, where the essential oil from *R. officinalis* presented only 1.64% of antioxidant activity. Constituents that have structures of an intermediate polarity, such as the aldehyde group, (-CHO), provide some hydrophobicity and exhibit large antioxidant activities in the β-carotene/linoleic acid system. They are more effective in protecting linoleic acid because they are more highly concentrated in the lipid phase [32].

A significant difference in antioxidant activity of the essential oils from the control at all concentrations was observed with the use of the ABTS method (Table 5), the control being more effective. A significant difference in the effects of the essential oils from *M. piperita*, *R. officinalis* and *P. boldus* was only observed at the concentration of 500 μg·mL^{-1}, whereas no statistical difference between the concentrations was observed for the *C. citratus* and *F. vulgare* oils.

Table 5. Percentages of antioxidant activity of the essential oils and BHT standard using the ABTS method.

Concentration (μg·mL^{-1})	Antioxidant Activities (%)					
	BHT	*M. piperita*	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
25	16.00gA	1.29bB	0.16aB	0.64bB	0.22bB	0.00aB
50	30.10fA	0.68bB	0.90aB	0.53bB	0.61bB	0.00aB
100	50.60eA	1.25bB	0.77aB	0.43bB	0.78bB	0.00aB
150	64.80dA	0.11bB	0.43aB	0.83bB	0.85bB	0.00aB
200	72.20cA	0.96bB	0.35aB	1.63bB	0.91bB	0.00aB
250	81.00bA	1.13bB	0.00aC	1.68bB	1.18bB	0.00aC
500	93.60aA	2.83aC	1.56aD	4.55aB	4.57aB	0.00aE

Means followed by the same lower case letter in the columns and the same upper case letter in the row do not differ significantly by the Scott-Knott test ($p \leq 0.05$).

According to Singh, Shushni and Belkheir [33], a high antioxidant potential (approximately 90% inhibition) was observed for the essential oil from *M. piperita*. According to the authors, the essential oil from *M. piperita* is composed of menthol, menthone, menthofuran and menthyl acetate. The authors emphasized the importance of natural products with an antioxidant potential for the food, cosmetic and pharmaceutical industries. The essential oils can be used as possible substitutes for synthetic products because they can prevent oxidation of food and can be used in the production of pharmaceuticals and cosmetics that remove free radicals present in the body.

Jordan *et al.* [34] stated that the essential oil from *R. officinalis* has a significant antioxidant potential for radical capture methods like the ABTS method. The authors report that essential oils with significant levels of γ-terpinene, *α*-terpinene, terpinolene and caryophyllene oxide present a significant activity. According to the authors, γ-terpinene, *α*-terpinene, terpinolene and caryophyllene oxide were not identified as the principal constituents, but they were present in concentrations sufficient for the capture of radical. However, these components were not identified in the present study. This fact explains the low activity observed because the principal constituents (1,8-cineol, *α*-pinene and camphor) are not capable of donating hydrogen atoms for the stabilization of ABTS.

The percentage of antioxidant activity of the essential oils measured by the hydroxyl method is presented in Table 6. The essential oil from *M. piperita* exhibited the highest activity (62.8%) at a concentration of 500 μg·mL^{-1}, followed by *R. officinalis* (46.2%), *C. citratus* (42.2%), *F. vulgare* (31.3%) and *P. boldus* (25.8%). According to the literature, the standard to be used would be mannitol; however, a low antioxidant activity, approximately 20% at the highest concentration, was observed for this compound.

Mimica-Dukic *et al.* [35] studied the essential oils from *M. piperita*, *M. longifolia* and *M. aquatica*. Greater activity was observed for the essential oil from *M.*

Table 6. Percentage of antioxidant activity of the essential oils and the mannitol standard by the hydroxyl method.

Concentration ($\mu g \cdot mL^{-1}$)	Antioxidant Activities (%)					
	Mannitol	*M. piperita*	*C. citratus*	*R. officinalis*	*P. boldus*	*F. vulgare*
25	0.00cD	28.70cA	22.40dB	10.30cC	0.00bD	0.75cD
50	0.00cC	40.70bA	11.70dB	13.60cB	0.00bC	0.00cC
100	0.00cC	42.90bA	11.30cB	12.30cB	0.00bC	0.00cC
150	1.52cC	26.60cA	16.60bB	16.20cB	2.92bC	3.41cC
200	3.76cC	31.00cA	19.90bB	27.20bA	3.00bC	4.15cC
250	9.44bD	42.10bA	24.40bC	31.20bB	6.11bD	10.80bD
500	19.70aE	62.80aA	42.20aB	46.20aB	25.80aD	31.30aC

Means followed by the same lower case letter in the columns and the same upper case letter in the row do not differ significantly by the Scott-Knott test ($p \leq 0.05$).

piperita (24.0%), which is composed of menthol (40.0%), methyl acetate (10.0%) and menthone (9.0%). The constituents identified in this study were not the same as those found by those authors, which may explain the difference in activity observed via the hydroxyl method**.** Essential oils that possess components capable of stabilizing the OH radical will not attack deoxyribose, and consequently, malondialdehyde will not be formed and no reaction with TBA will occur.

The greatest activity was observed for the essential oils from *C. citratus* and *P. Boldus* (Table 7) when the molybdenum reduction method was employed. It is noted that 1.54 and 1.82 μg of the essential oils from *C. citratus* and *P. Boldus*, respectively, were equivalent to 1 g of ascorbic acid, whereas it required 20 μg of the essential oil from *F. vulgare* to obtain the same equivalence.

Silva *et al.* [36] evaluated the antioxidant activity of the essential oil from the plants of the Lamiaceae family (*Mentha pulegium* and *Mentha viridis*) by the molybdenum reduction method. They found that a higher activity was observed for the essential oil from *M. pulegium* (50.0% of pulegone, 31.9% menthol and 16.6% menthone) than for the standard used (BHT), followed by the essential oil from *M. viridis*, which is composed of 40.7% linalool, 13.9% carvone and 8.6% *α*-terpinene. The data do not corroborate those found in this study because no significant activity was observed for the essential oil from *M. piperita* using the molybdenum reduction method. The different compositions of the essential oils may have influenced the results.

No antioxidant activity for the essential oils (*M. piperita, C. citratus*, *R. officinalis*, *F. vulgare* and *P. boldus*) was observed by the reducing power method; that is, no reduction of the iron ion occurred. Silva *et al.* [36] observed a limited activity in reducing the iron ion for the essential oil from *M. viridis.* However, the activity observed for the essential oil from *M. pulegium* at the concentrations of 0.78 to 50 $\mu L \cdot mL^{-1}$ exceeded that of the BHT standard. Although the essential oils are from plant species of the same genus and family, they may contain dif-

Table 7. Antioxidant activity of the essential oils determined by the phosphomolibdenum reduction method.

Plant	Phosphomolybdenum Method
	Amount of essential oil equivalent to one μg of ascorbic acid (μg)
M. piperita	5.3
C. citratus	**1.54**
R. officinalis	5.57
P. boldus	**1.82**
F. vulgare	20.66

ferent components and, consequently, exhibit different antioxidant activities. Teixeira *et al.* [29] evaluated the antioxidant properties of the essential oil from *R. officinalis* by the reducing power method and observed an activity lower than the equivalent of 5 micromoles of ascorbic acid per gram of essential oil. No such activity was observed in the present study.

4. Conclusion

The principal constituents found in the essential oil from *M. piperita* were carvone and limonene. Geranial, neral and myrcene were found in the essential oil from *C. citratus*. Camphor, 1,8-cineole, and *α*-pinene were found in the essential oil from *R. officinalis*. The essential oil from *P. boldus* contained *α*-terpinyl formate, *p*-cymene, 1,8-cineole, ascaridol and terpinen-4-ol, and the essential oil from *F. vulgare* contained methyl chavicol, limonene and fenchone. In the evaluation of the antibacterial activity, *S. aureus* was more sensitive to the oil from *P. boldus*. *Listeria monocytogenes* and *Salmonella* choleraesuis were sensitive to the essential oil from *C. citratus*. *Escherichia coli* was affected by the essential oils from *C. citratus* and *P. boldus*, whereas *Pseudomonas aeruginosa* was resistant to all the oils tested. All the essential oils tested presented low or no antioxidant activities with the radical sequestration methods (DPPH and ABTS). They were not effective in protecting against the oxidation of linoleic acid. They were effective only in the protection of deoxyribose, where the peppermint oil presented the highest antioxidant activity. The activities of the essential oils were greater than those of the standard (mannitol). In the molybdenum reduction method, the essential oils from *C. citratus* and *P. boldus* were more effective in reducing molybdenum than the other oils, and no antioxidant activity was observed by the reducing power method.

Acknowledgements

The authors acknowledge the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) in the form of a PVNS fellowship, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

References

[1] Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A. and Petrovick, P.R. (2007) Farmacognosia: da planta ao medicamento. 6th Edition, UFSC/UFRGS, Porto Alegre, 1102 p.

[2] Miranda, C.A.S.F., Cardoso, M.G., Machado, S.M.F., Gomes, M.S., Andrade, J. and Teixeira, M.L. (2014) Correlação entre composição química e eficácia antioxidante de óleos essenciais de plantas condimentares por Análise de Agrupamentos Hierárquicos (HCA). *E-xacta*, **7**, 65-74. https://doi.org/10.18674/exacta.v7i1.1223

[3] Gomes, M.S., Cardoso, M.G., Soares, M.J., Batista, L.R., Machado, S.M.F., Andrade, M.A., Azeredo, C.M.O., Resende, J.M.V. and Rodrigues, L.M.A. (2014) Use of Essential Oils of the Genus Citrus as Biocidal Agents. *American Journal of Plant Sciences*, **5**, 299-305. https://doi.org/10.4236/ajps.2014.53041

[4] Teixeira, M.L., Cardoso, M.G., Souza, P.E., Machado, S.M.F., Andrade, M.A., Gomes, M.S. and Andrade, J. (2012) Citrumelo Swingle: Caracterização Química, Atividade Antioxidante e Antifúngica dos óleos Essenciais das Cascas Frescas e Secas. *Magistra*, **24**, 194-203.

[5] Adams, R.P. (2007) Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscop. 4th Edition, Allured, Carol Streem, 804 p.

[6] Van Den Dool, H. and Kratz, P.D. (1963) A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. *Journal Chromatography A*, **11**, 463-471. https://doi.org/10.1016/S0021-9673(01)80947-X

[7] National Committee for Clinical Laboratory Standards (2003) Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically: Approved Standard. 6nd Edition, Wayne (NCCLS Document M7-A6; NCCLS, 940).

[8] OgunwAnde, I.A., OLawore, N.O., Ekundayo, O., Walker, T.M., Schmidt, J.M. and Setzer, W.N. (2005) Studies on the Essential Oils Composition, Antibacterial and Cytotoxicity of *Eugenia uniflora* L. *International Journal of Aromatherapy*, **15**, 152-147. https://doi.org/10.1016/j.ijat.2005.07.004

[9] Guerrini, A., Sacchetti, G., Rossi, D., Paganetto, G., Muzzoli, M., Andreotti, E., Tognolini, M., Maldonado, M.E. and Bruni, R. (2009) Bioactivities of *Piper aduncum* L. and *Piper obliquum* Ruiz & Pavon (Piperaceae) Essential Oils from Eastern Ecuador. *Environmental Toxicology and Pharmacology*, **27**, 39-48. https://doi.org/10.1016/j.etap.2008.08.002

[10] Lima, R.K., Cardoso, M.G., Andrade, M.A., Guimarães, P.L., Batista, L.R. and Nelson, D.L. (2012) Bactericidal and Antioxidant Activity of Essential Oils from *Myristica fragrans* Houtt and *Salvia microphylla*. *Journal of the American Oil Chemists' Society*, **89**, 523-528. https://doi.org/10.1007/s11746-011-1938-1

[11] Lopes-Lutz, D., Alviano, D.S., Alviano, C.S. and Kolodziejczyk, P.P. (2008) Screening of Chemical Composition, Antimicrobial and Antioxidant Activities of Artemisia Essential Oils. *Phytochemistry*, **69**, 1732-1738. https://doi.org/10.1016/j.phytochem.2008.02.014

[12] Guerreiro, A.C., Gago, C.M.L., Miguel, M.G.C. and Antunes, M.D.C. (2013) The Effect of Temperature and Film Covers on the Storage Ability of *Arbutus unedo* L. Fresh Fruit. *Scientia Horticulturae*, **159**, 96-102. https://doi.org/10.1016/j.scienta.2013.04.030

[13] Boulanouar, B., Abdelaziz, G., Aazza, S., Gago, C. and Miguel, M.G. (2013) Antioxidant Activities of Eight Algerian Plant Extracts and Two Essential Oils. *Industrial Crops and Products*, **16**, 85-96. https://doi.org/10.1016/j.indcrop.2013.01.020

[14] Kanatt, S.R., Chander, R. and Sharma, A. (2007) Antioxidant Potential of Mint (*Mentha spicata* L.) in Radiation-Processed Lamb Meat. *Food Chemistry*, **100**, 451-458. https://doi.org/10.1016/j.foodchem.2005.09.066

[15] Prieto, P., Pineda, M.E. and Aguilar, M. (1999) Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, **269**, 337-341. https://doi.org/10.1006/abio.1999.4019

[16] Ferreira, D.F. (2011) Sisvar: A Computer Statistical Analysis System. *Ciência e Agrotecnologia*, **35**, 1039-1042.

[17] Evrendilek, G.A. (2015) Empirical Prediction and Validation of Antibacterial Inhibitory Effects of Various Plant Essential Oils on Common Pathogenic Bacteria. *International Journal of Food Microbiology*, **202**, 35-34. https://doi.org/10.1016/j.ijfoodmicro.2015.02.030

[18] Gobbo-Neto, L. and Lopes, N.P. (2007) Plantas Medicinais: Fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, **30**, 374-381. https://doi.org/10.1590/S0100-40422007000200026

[19] Miranda, C.A.S.F., Cardoso, M.G., Carvalho, M.L.M., Machado, S.M.F., Gomes, M.S., Andrade, J. and Teixeira, M.L. (2015) Atividade alelopática de óleos essenciais de plantas medicinais na germinação e vigor de aquênios de alface. *Semina*: *Ciências Agrárias*, **36**, 1783-1798. https://doi.org/10.5433/1679-0359.2015v36n3supl1p1783

[20] Lemos, M.F., Lemos, M.F, Pacheco, H.P., Endringer, D.C. and Scherer, R. (2015) Seasonality Modifies Rosemary's Composition and Biological Activity. *Industrial Crops and Products*, **70**, 41-47. https://doi.org/10.1016/j.indcrop.2015.02.062

[21] Vogel, H., Razmilic, I., Muñoz, P., Doll, U. and Martín, J.S. (1999) Studies of Genetic Variation of Essential Oil and Alkaloid Content in Boldo (*Peumus boldus*). *Planta Médica*, **65**, 90-99. https://doi.org/10.1055/s-2006-960450

[22] Vila, R., Valenzuela, L., Bello, H., Canigueral, S., Montes, M. and Adzet, T. (1999) Composition and Antimicrobial Activity of the Essential Oil of *Peumus boldus* Leaves. *Planta Médica*, **65**, 178-179. https://doi.org/10.1055/s-2006-960461

[23] Burt, S. (2004) Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. *International Journal of Food Microbiology*, **94**, 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[24] Valeriano, C., Piccoli, R.H., Cardoso, M.G. and Alves, E. (2012) Atividade antimicrobiana de óleos essenciais em bactérias patogênicas de origem alimentar. *Revista Brasileira de Plantas Medicinais, Botucatu*, **14**, 57-67. https://doi.org/10.1590/S1516-05722012000100009

[25] Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H.C. and Demirci, F. (2002) Antimicrobial Screening of *Mentha piperita* Essential Oils. *Journal of Agricultural and Food Chemistry*, **50**, 3943-3946. https://doi.org/10.1021/jf011476k

[26] Silva, F.L., Sugauara, E.Y.Y., Magalhães, H.M., Pascotto, C.R., Colauto, N.B., Linde, G.A. and Gazim, Z.C. (2014) Atividade antimicrobiana do óleo essencial de Cymbopogon citratus. *Arquivo de Ciências Veterinárias e Zoologia da UNIPAR*, **17**, 179-182.

[27] Yadegarinia, D., Gachkar, L., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A. and Rasooli, I. (2006) Biochemical Activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. Essential Oils. *Photochemistry*, **67**, 1161-1298. https://doi.org/10.1016/j.phytochem.2006.04.025

[28] Guimarães, L., Cardoso, M.G., Sousa, E., Andrade, J. and Vieira S.S. (2011) Atividades antioxidante e fungitóxica do óleo essencial de capim-limão e do citral.

Revista Ciência Agronômica, **42**, 464-472.
https://doi.org/10.1590/S1806-66902011000200028

[29] Teixeira, B., Marques, A., Ramos, C., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A. and Nunes, M.L. (2013) Chemical Composition and Antibacterial and Antioxidant Properties of Commercial Essential Oils. *Industrial Crops and Products*, **43**, 587-595. https://doi.org/10.1016/j.indcrop.2012.07.069

[30] Roby, M.H.H., Sharhan, M.A., Selim, K.A.H. and Khalel, K.I. (2013) Antioxidant and Antimicrobial Activities of Essential Oil and Extracts of Fennel (*Fóleo essencialniculum vulgare* L.) and Chamomile (*Matricaria chamomilla* L.). *Industrial Crops and Products*, **44**, 437-445. https://doi.org/10.1016/j.indcrop.2012.10.012

[31] Gachkar, L., Yadegarinia, D., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A. and Rasooli, I. (2007) Chemical and Biological Characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* Essential Oils. *Food Chemistry*, **2**, 898-904.
https://doi.org/10.1016/j.foodchem.2006.06.035

[32] Kulisic, T., Radonic, A., Katalinic, V. and Milos, M. (2004) Use of Different Methods for Testing Activity of Oregano Essential Oil. *Food Chemistry*, **85**, 633-640.
https://doi.org/10.1016/j.foodchem.2003.07.024

[33] Singh, R., Shushni, M.A.M. and Belkheir, A. (2015) Antibacterial and Antioxidant Activities of *Mentha piperita* L. *Arabian Journal of Chemistry*, **8**, 322-328.
https://doi.org/10.1016/j.arabjc.2011.01.019

[34] Jordan, M.J., Lax, V., Rota, M.C., Loran, S. and Sotomayor, J.A. (2013) Effect of the Phenological Stage on the Chemical Composition, and Antimicrobial and Antioxidant Properties of *Rosmarinus officinalis* L. Essential Oil and Its Polyphenolic Extract. *Industrial Crops and Products*, **48**, 144-152.
https://doi.org/10.1016/j.indcrop.2013.04.031

[35] Mimica-Dukić, N., Bozin, B., Soković, M., Mihajlović, B. and Matavulj, M. (2003) Antimicrobial and Antioxidant Activities of Three *Mentha* Species Essential Oil. *Planta Medica*, **69**, 413-419. https://doi.org/10.1055/s-2003-39704

[36] Silva, L.F., Cardoso, M.G., Batista, L.R., Gomes, M.S., Rodrigues, L.M.A., Rezende, D.A.C.S., Teixeira, M.L., Carvalho, M.S.S., Santiago, J.A. and Nelson, D.L. (2015) Chemical Characterization, Antibacterial and Antioxidant Activities of Essential Oils of *Mentha viridis* L. and *Mentha pulegium* L. (L). *American Journal of Plant Sciences*, **6**, 666-675. https://doi.org/10.4236/ajps.2015.65072