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FENÓLICOS TOTAIS E POTENCIAL ANTIOXIDANTE DE CINCO ESPÉCIES DA FAMÍLIA LAMIACEAE

TOTAL PHENOL AND ANTIOXIDANT POTENTIAL OF FIVE SPECIES OF LAMIACEAE FAMILY

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RESUMO

A família Lamiaceae tem distribuição cosmopolita, incluindo cerca de 300 gêneros e 7500 espécies. Numerosas doenças têm sido relacionadas com a superprodução de radicais livres. A capacidade antioxidante está relacionada com compostos capazes de proteger um sistema biológico contra o efeito potencialmente prejudicial de processos ou reações envolvendo espécies reativas de oxigênio e nitrogênio. No presente estudo, os extratos etanólicos, obtidos por maceração de *Melissa officinalis*, *Mentha* sp., *Ocimum basilicum*, *Plectranthus barbatus* e *Rosmarinus officinalis*, foram avaliados quanto à atividade antioxidante, tiveram determinados os teores de compostos fenólicos e identificados os compostos voláteis por Cromatografia Gasosa acoplada à Espectrometria de Massas (CG-EM). Os extratos etanólicos de *M. officinalis*, *Mentha* sp., *P. barbatus* e *R. officinalis* foram capazes de reduzir o radical livre, DPPH, com CE_{50} de 3,81, 11,89, 6,92 e 11,54 µg/ml, respectivamente, apresentando melhor atividade que o BHT, utilizado como padrão. No entanto, o extrato etanólico de *O. basilicum*, apresentou baixa atividade e alto valor de CE_{50}. Os fenóis totais de todos os extratos variaram de 85,18 a 212,74 µg/ml. O maior teor de compostos fenólicos foi detectado no extrato etanólico de *M. officinalis*. Análise dos compostos voláteis mostrou a presença de fitol em todos os extratos, sendo predominante em *P. barbatus* and *R. officinalis*, e ainda nonadecano e heptadecano identificados em *M. officinalis*. Frações obtidas das cinco espécies de Lamiaceae também apresentaram efeito antioxidante. Nas frações mais ativas foram detectados principalmente compostos fenólicos, como flavonoides, taninos e cumarinas. Esses resultados incentivam novos estudos com essas espécies, no sentido de se isolar os compostos responsáveis pela atividade antioxidante, para que, no futuro, possam ser usadas como antioxidantes de origem natural.

Palavras-chave: *Lamiaceae, antioxidante, compostos fenólicos.*

ABSTRACT

Lamiaceae family has a cosmopolitan distribution, including about 300 genus and 7500 species. Numerous diseases have been related to free radicals overproduction. Antioxidant capacity is related to compounds capable of protecting a biological system against the potentially harmful effect of processes or reactions involving reactive oxygen and nitrogen species. In the present study, the ethanol extracts, obtained by maceration of *Melissa officinalis*, *Mentha* sp., *Ocimum basilicum*, *Plectranthus barbatus,* and *Rosmarinus officinalis* were evaluated for antioxidant activity, the total phenolic content was determined, and volatile compounds were identified by Gas Chromatography coupled to Mass Spectrometry (GC-MS). Ethanol extracts of *M officinalis*, *Mentha* sp., *P. barbatus,* and *R. officinalis* were able to reduce the free radical DPPH with IC_{50} of 3.81, 11.89, 6.92 and 11.54 µg/ml respectively, presenting better activity than the BHT, used as a standard. However, the ethanol extract of *O. basilicum* showed low activity and high IC_{50} value. Total phenols of all extracts ranged from 85.18 to 212.74 µg/ml. The highest phenolic concentration was observed in the ethanol extract of *M. officinalis*. Analysis of volatile compounds showed the presence of phytol in all extracts, being predominant in *P. barbatus* and *R. officinalis*, and also nonadecane and heptadecane in *M. officinalis*. Fractions obtained from the five species of Lamiaceae also presented antioxidant effect. In the most active fractions were

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mainly phenolic compounds, such as flavonoids, tannins, and coumarins. These results encourage new studies with these species in order to isolate the compounds responsible for this antioxidant activity so that in the future they can be used as natural antioxidants.

Keywords: *Lamiaceae, antioxidant, phenolics.*

1. INTRODUCTION

Reactive oxygen species (ROS) damage DNA or oxidize lipids and proteins. This process is harmful to live organisms and may play an important role in coronary heart disease, atherosclerosis, cancer, and the aging process (Marinova and Yanishilieva, 2003). Clinical trials and epidemiological studies have established an inverse correlation between dietary utilization of fruits, vegetables and teas and the occurrence of diseases related to oxidative stress (Droge, 2002). Thus, it is of great interest, from the medicinal and nutritional point of view, the knowledge of the antioxidant capacity of constituents of foods that are consumed.

The Lamiaceae family has a cosmopolitan distribution. In Brazil, there are 26 genera with approximately 350 species. Because they are aromatic, these plants are used as a condiment, or as often teas, such as lemon balm (*Melissa officinalis* L.), mint (*Mentha sp*.), basil (*Ocimum basilicum*), brazilian-boldo (*Plectranthus barbatus*), and rosemary (*Rosmarinus officinalis*) (Lorenzi and Sousa, 2005). It is well-known that many spices, especially those belonging to the Lamiaceae family possess a wide range of biological and pharmacological activities. Some of them are specifically related to the essential oils like antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, and anticarcinogenic activities (Araújo *et al*., 2014; Bozin *et al*., 2006).

This work is focused on the evaluation of the antioxidant activity of the ethanol extracts of Lamiaceae species.

2. MATERIALS AND METHODS

Chemicals

All solvents PA were purchased from Vetec (Brazil); 2,6-di-tert-butyl-4-methylphenol (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (AA), tannic acid and Folin-Dennis reagent were obtained from Sigma (St. Louis, USA).

Plant material and extraction

The Lamiaceae species were collected in Carmópolis de Minas, Minas Gerais, Brazil (20º32'44.57'"S and 44º38'37.09"W), in April 2011. The vouchers specimens were identified by Dr. Alexandre Salino and deposited at the Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil (*Melissa officinalis* BHCB 147242, *Mentha sp.* BHCB 147244, *Ocimum basilicum* BHCB 147240*, Plectranthus barbatus* BHCB 147241*,* and *Rosmarinus officinalis* BHCB 147245). The present study had been approved by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through Access and Shipment Component of Genetic Heritage for scientific research purpose (no. 010655/2011-5) and Certificate of registration for collection of botanical, fungal and microbiological material in SISBIO (no. 30006-1), Register IBAMA (no. 5282691).

The fresh plant material was extracted by cold maceration in ethanol PA for a period of 10 days at room temperature. After it was filtrated and concentrated in a rotary evaporator at 40 °C under reduced pressure and lyophilized to yield ethanol extracts (Araújo *et al*., 2014). Part of these extracts (1.0 g) were dissolved in 100 ml of EtOH/H_2O (7:3) and then partitioned successively with 50 ml of hexane, dichloromethane, ethyl acetate and butanol (three times with each solvent), resulting in hexane (Hex), dichloromethane (DCM), ethyl acetate (EA), butanol (But) and hydroethanol (HE) fractions (Table 1).

Determination of total phenolic content

The total phenolic content of ethanol extracts obtained from Lamiaceae species was determined using the Folin-Dennis reagent with tannic acid as the standard (AOAC, 1995). The results were expressed as tannic acid equivalents/ml.

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DPPH radical scavenging assay

The radical-scavenging abilities of Lamiaceae species were based on reactions with a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and were compared to the standard, 2,6-di-tert-butyl-4-methylphenol (BHT). The determination of antioxidant activity using the DPPH method was adapted for use with microplates (Araújo *et al*., 2013). A DPPH solution (0.002% w/v) was prepared in ethanol. Exactly 75 µl of the samples or standards (1, 10, 100, 250 and 500 µg/ml) were added to the wells in a 96-well flat-bottom plate containing 150 µl of DPPH solution. The plate was then covered and left in the dark at room temperature (25 °C). After 30 min, the absorbance at 517 nm was measured with a spectrophotometer (Biotek Power Wave XS2, USA), and ethanol was used for the baseline correction.

Scavenging ability was expressed as the inhibition percentage and was calculated by equation (Burda and Oleszek, 2001):

$$\text{Scavenging ability } (\%) = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control} \times 100} \times 100 \quad \text{(Eq. 1)}$$

where $Abs_{control}$ is the absorbance of the DPPH radical in ethanol and Abs_{sample} is the absorbance of samples and standards in ethanol + DPPH. The antioxidant activity of all of the samples was expressed as IC_{50}, which was defined as a concentration (in µg/ml) of samples required to inhibit the formation of the DPPH radicals by 50%. IC_{50} values were calculated using the Probit analysis (Finney, 1980). All assays were performed in triplicate.

Analysis by GC-MS

The volatile compounds from the ethanol extracts were investigated by gas chromatography/mass spectrometry (GC/MS) (Araújo *et al*., 2014). The compounds were identified by mass spectral database search (NIST) and showed mass spectra with match factors ≥ 90%.

Phytochemical analysis

The fractions were qualitatively screened for the presence of different classes of natural products such as alkaloids, steroids, triterpenoids, coumarins, tannins, saponins and flavonoids (Matos, 1997; Wagner *et al*., 2001).

Statistical Analysis

Student's t-test was utilized to evaluate the statistical difference between the control group and the group exposed to ethanol extracts. A P value < 0.05 was considered statistically significant. The analyses were performed using the GraphPad software.

3. RESULTS AND DISCUSSION:

The ethanol extracts obtained from Lamiaceae species were examined for their radical-scavenging ability by DPPH method, and the results are presented in Figure 1. Their IC_{50} values are presented in Figure 2. Analysis of Figure 1 showed an antiradical scavenger effect of the ethanol extracts dose-dependent as well as those presented by AA and BHT. Thus, despite the fact that the scavenging activity of AA was greater than those of ethanol extracts, at 10 µg/ml. Antioxidant activities of all extracts, standard BHT and AA are presented in Figure 2. Ethanol extracts of *M. officinalis*, *Mentha sp*., *P. barbatus,* and *R. officinalis* were able to reduce the radical DPPH, with an IC_{50} of 3.81, 11.89, 6.92 and 11.54 µg/ml, respectively, a better radical-scavenging activity than did BHT, a commercial antioxidant (IC_{50} = 16.36 µg/ml), contrasting with the ethanol extract of *Ocimum basilicum*, which presented low activity (and high IC_{50}). All extracts were less active than AA, with an IC_{50} 1.62 µg/ml.

In this study, the ethanol extracts of *M. officinalis* and *P. barbatus* exhibited greater antioxidant activity than the other extracts. It may be explained by the presence of phenolic compounds in the ethanol extracts. The amounts of total phenolic compounds in all extracts were also investigated shown in Figure 3. The total phenolic compounds of all extracts ranged from 85.18 to 212.74 µg/ml. The highest phenolic concentration was observed in the ethanol extract of *M. officinalis* followed by the *P. barbatus*, *R. officinalis*, *Mentha sp*. and *O. basilicum*. The IC_{50} values determined for the extracts could be correlated with the total phenolic compounds.

Phenolic compounds, such as flavonoids, have high antioxidant potential and strong free radical scavenging activity and are much more efficient than the vitamins C and E in protecting cells from free radical damage (Vinson *et al*.,

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1995; Wiseman *et al*., 1997).

The ethanol extracts from five species tested in this work were analyzed by gas chromatography/mass spectrometry (GC/MS) (Araújo *et al*., 2014). Among the main compounds identified, only phytol was present in all species studied (Araújo *et al*., 2014), being a predominant component of *P. barbatus* and *R. officinalis*. Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a diterpenoid particularly interesting due the several biological activities reported. Phytol was evaluated for antioxidant activity using non- and pre-clinical models, with potential effect, being canned be a good candidate for the development of treatments of oxidative stress-mediated diseases (Costa *et al*., 2016).

Nonadecane and heptadecane found in *M. officinalis* also exhibited the antioxidant effect. Essential oil of *Acacia cyclops* showed high antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Nonadecane was identified as the major constituent of this essential oil (Jelassi *et al*., 2017). Heptadecane demonstrated a potent anti-oxidative activity by protecting the endothelial cell line from tert-butylhydroperoxide induced oxidative stress (Kim *et al*., 2013).

The fractions of Lamiaceae species also were evaluated by DPPH assay (Table 2) and exhibited an antiradical scavenger effect dose-dependent. The fractions of *R. officinalis* were the most active among the fractions tested. The But fraction had an IC_{50} value similar to the value obtained for AA. The other fractions showed IC_{50} values smaller or equivalent to those found for BHT.

The EA and But fractions of *M. officinalis* showed highest antioxidant action, with effect compared to AA and being more effective than the ethanol extract. The DCM and HE fractions showed a similar effect to BHT.

The fractions obtained from *P. barbatus* were less active than the ethanol extract that gave rise to them. Only DCM and HE fractions had action compared to that of BHT.

The But fraction of *Mentha* sp. was the most effective of this species, being more active than the ethanol extract. The DCM, EA and HE fractions exhibited free radical-scavenging activity similar to BHT.

The ethanol extract of *O. basilicum* was the least effective extract. However, the EA and DCM fractions presented IC_{50} values similar to those obtained for AA and BHT, respectively. The But fraction was also much more effective than BHT, with IC_{50} = 2.56 μg/ml.

Phytochemical analyses suggested the presence of steroids/triterpenes, flavonoids, coumarins, alkaloids, tannins and saponins in the fractions from Lamiaceae species employed in this study. However, in the fractions with better antioxidant effects (EA and But fractions of *M. officinalis* and *O. basilicum* and But a fraction of *R. officinalis*), which had IC_{50} values between 1.03 and 2.56 μg/ml, were detected principally phenolic compounds, such as flavonoids, tannins, and coumarins.

Flavonoids are widely distributed in plants and represent important antioxidants compounds, being the flavones and catechins the most powerful flavonoids in this sense (Saxena *et al*., 2012). Flavonoids in aglycones form are also antioxidant more potent than their corresponding glycosides (Kumar and Pandey, 2013).

The antioxidant activity of flavonoids will depend on the arrangement of functional groups in their structure. The configuration, substitution and a total number of hydroxyl groups considerably influence many mechanisms of antioxidant action, such as the elimination of free radicals and the chelation capacity of metal ion (Kelly *et al*., 2002; Pandey *et al*., 2012).

Tannins are a class of secondary metabolites found in several species of vegetables and have been considered "health-promoting" components in plant-derived foods and beverages. Tannins have antioxidant action, but they do not only act as primary antioxidants (i.e., they are capable of donating electrons or hydrogen atoms), they also function as secondary antioxidants. Tannins have the ability to chelate metal ions forming complexes with them, retard oxidation and inhibit lipid peroxidation via the cyclooxygenase pathway (Amarowicz, 2007; Zhang *et al*., 2004).

Coumarins, substances widely distributed in nature, have been shown to be a potential source of antioxidants, is found in green plants, in fruits, green tea, and other foods and spices. They have been described as compounds that reduce the formation and stimulate the scavenging of reactive oxygen species (ROS), exhibiting protective antioxidative effect against tissue damage (Bubols *et al*., 2013).

4. CONCLUSIONS:

The ethanol extracts had high antioxidant potential, since *M. officinalis*, *Mentha* sp., *P. barbatus,* and *R. officinalis* were more active than

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the BHT, used as a reference compound. The ethanol extract of *M. officinalis* presented a higher amount of phenolic compounds. The fractions of Lamiaceae species also present antioxidant effect. In the fractions more active (EA and But fractions of *M. officinalis* and *O. basilicum* and But a fraction of *R. officinalis*) were detected principally phenolic compounds, such as flavonoids, tannins, and coumarins. These results encourage further studies on these species in order to isolate and identify the compounds responsible for antioxidant activities, and may in the future be used as naturally occurring antioxidants.

5. ACKNOWLEDGMENTS

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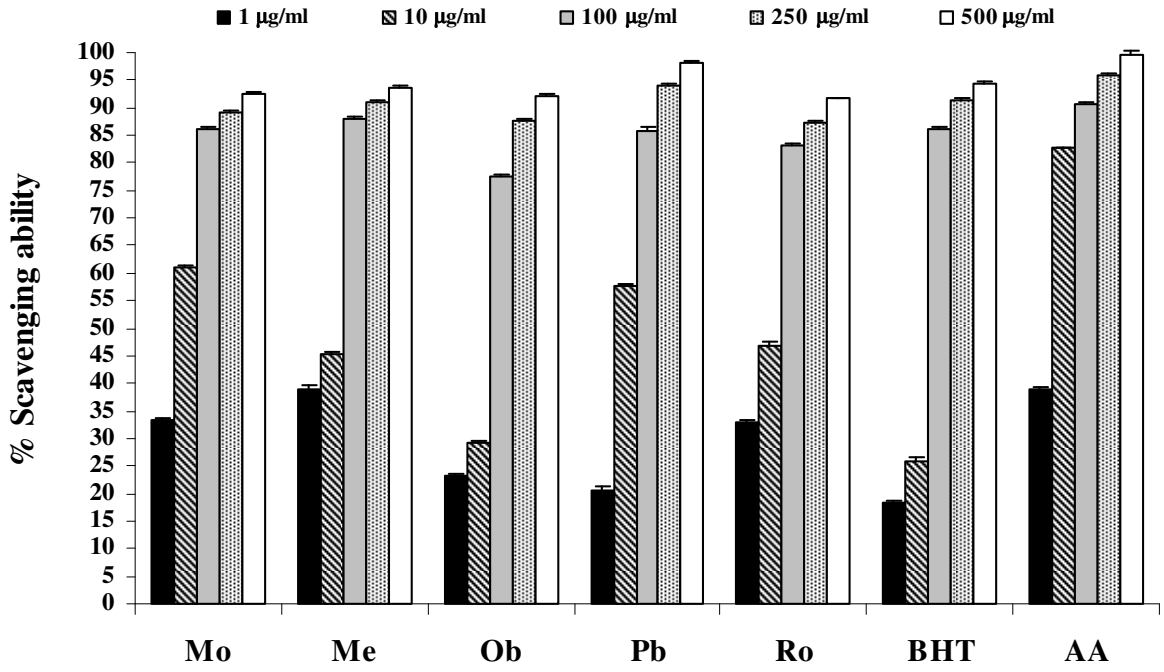

Figure 1. *DPPH radical-scavenging ability of ethanol extracts at five different concentrations. Values represent averages ± standard deviations for triplicate experiments.*

Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus, Ro: Rosmarinus officinalis, BHT: 2,6-di-tert-butyl-4-methylphenol and AA: ascorbic acid.

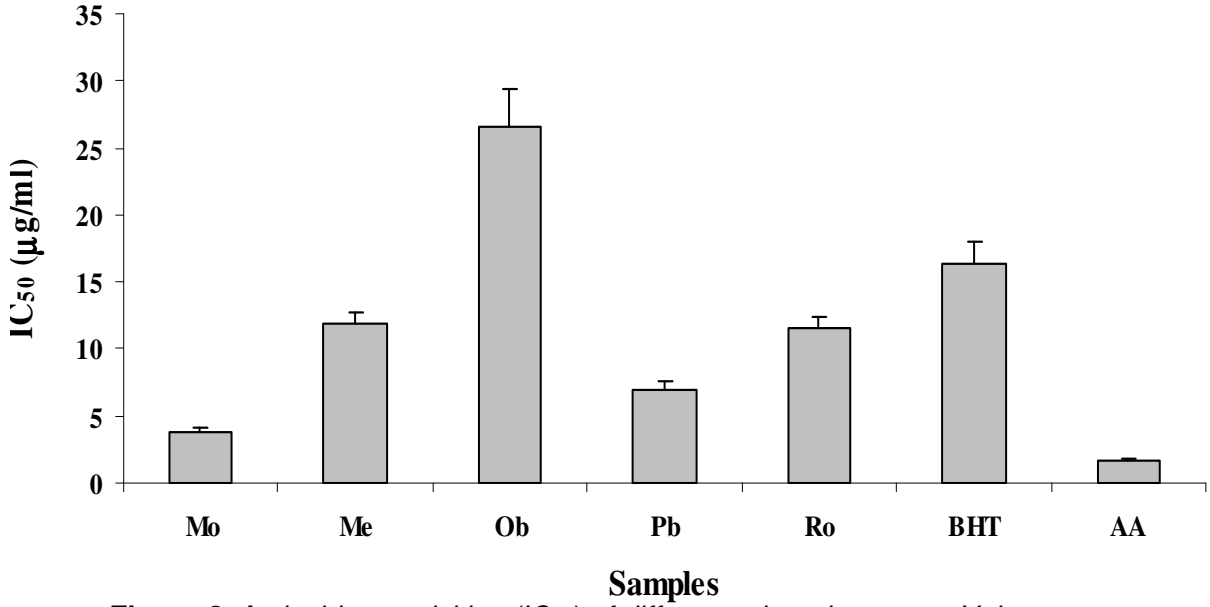

Figure 2. *Antioxidant activities (IC_{50}) of different ethanol extracts. Values represent averages ± standard deviations for triplicate experiments.*

Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus, Ro: Rosmarinus officinalis, BHT: 2,6-di-tert-butyl-4-methylphenol and AA: ascorbic acid.

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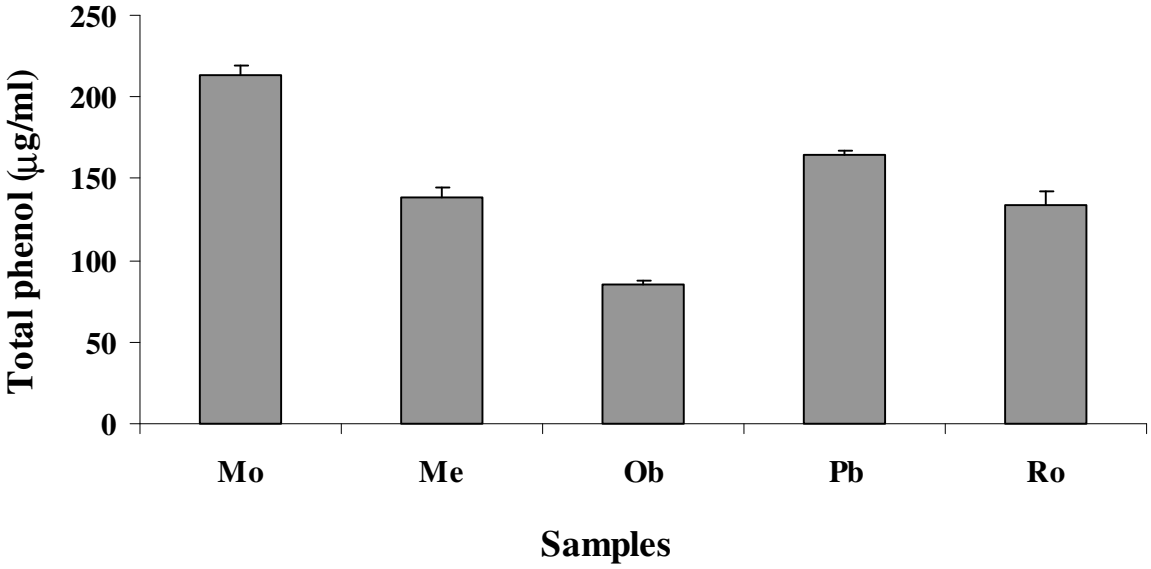
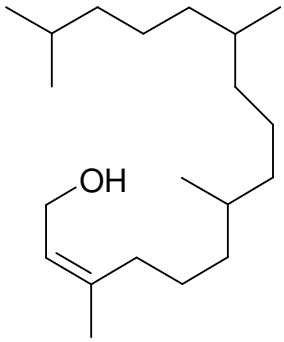
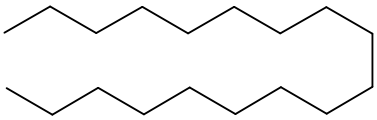


Figure 3. *Total phenols of different ethanol extracts. Values represent averages ± standard deviations for triplicate experiments.*

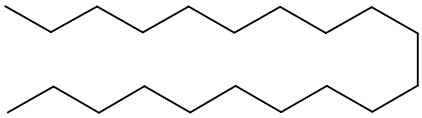
Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus and Ro: Rosmarinus officinalis.



Phytol (1)



Heptadecane (2)



Nonadecane (3)

Figure 4. *Structures of compounds identified by gas chromatography/mass spectrometry obtained from ethanol extracts of five species of Lamiaceae family.*

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Table 1. *Masses obtained for the ethanol extract and fractions of Melissa officinalis, Mentha sp., Ocimum basilicum, Plectranthus barbatus, and Rosmarinus officinalis.*

Samples	Mass (g)						
	Fresh plant	Et	Hex	DCM	EA	But	HE
Mo	63.32	2.31	0.1946	0.1510	0.1186	0.1400	0.2323
Me	235.02	10.17	0.1841	0.1783	0.2172	0.1500	0.1702
Ob	303.11	2.78	0.1729	0.1690	0.1054	0.2180	0.2053
Pb	140.94	3.14	0.2870	0.2024	0.1914	0.1487	0.1308
Ro	106.84	6.30	0.1308	0.1071	0.1366	0.3201	0.1641

Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus and Ro: Rosmarinus officinalis.

Et: ethanol extract, Hex: hexane fraction, DCM: dichloromethane fraction, EA: ethyl acetate fraction, But: butanol fraction and HE: hydroethanol fraction.

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Table 2. DPPH scavenging activity and IC_{50} values of the ethanol extract and fractions of Lamiaceae species.

Samples	**DPPH-scavenging activity**					**IC_{50}**
Mo	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
Hex	41.29±0.27^{ab}	43.22±0.40^{ab}	46.23±0.23^{ab}	49.01±0.23^{ab}	54.73±0.58^{ab}	273.85±13.90^{ab}
DCM	40.13±0.27^{ab}	43.45±0.23^{ab}	68.32±0.48^{ab}	92.35±0.23^{ab}	94.36±0.27^{b}	17.69±2.67^{b}
EA	46.47±0.23^{ab}	85.86±0.23^{ab}	92.51±0.54^{ab}	94.36±0.13^{a}	97.06±0.13^{ab}	1.03±0.04^{a}
But	46.69±0.40^{ab}	70.26±0.58^{ab}	89.42±0.27^{ab}	91.81±0.13^{b}	94.28±0.13^{b}	1.34±0.18^{a}
HE	37.42±0.49^{ab}	42.59±0.25^{ab}	77.41±0.38^{ab}	89.65±0.25^{ab}	93.02±0.14^{ab}	15.43±1.83^{b}
Samples	**DPPH-scavenging activity**					**IC_{50}**
Me	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
Hex	35.25±0.18^{ab}	38.40±0.48^{ab}	45.13±0.63^{ab}	94.63±0.32^{a}	97.16±0.32^{ab}	104.37±4.45^{ab}
DCM	38.72±0.48^{a}	42.82±0.48^{ab}	74.98±0.18^{ab}	97.27±0.18^{ab}	99.68±0.32^{a}	16.40±1.49^{b}
EA	37.04±0.48^{ab}	43.03±0.18^{ab}	90.12±0.48^{a}	92.74±0.32^{ab}	95.37±0.18^{ab}	13.20±1.56^{b}
But	40.82±0.48^{ab}	54.80±0.18^{ab}	92.01±0.18^{ab}	93.90±0.18^{ab}	96.74±0.18^{ab}	3.09±0.16^{ab}
HE	34.05±0.57^{ab}	37.09±0.38^{ab}	79.38±0.75^{ab}	90.88±0.25^{b}	93.02±0.38^{ab}	18.92±1.76^{b}
Samples	**DPPH-scavenging activity**					**IC_{50}**
Ob	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
Hex	31.81±0.48^{ab}	37.81±0.48^{ab}	48.46±0.32^{ab}	62.37±0.55^{ab}	84.93±0.18^{ab}	67.54±4.01^{ab}
DCM	36.44±0.32^{ab}	40.87±0.32^{ab}	90.83±0.32^{a}	92.52±0.37^{ab}	96.21±0.55^{ab}	14.10±1.39^{b}
EA	40.02±0.73^{ab}	75.02±0.32^{ab}	92.83±0.37^{ab}	95.68±0.48^{a}	99.89±0.18^{a}	1.80±0.16^{a}
But	38.66±0.32^{a}	65.11±0.18^{ab}	92.62±0.18^{ab}	95.47±0.18^{a}	96.52±0.00^{ab}	2.56±0.24^{ab}
HE	23.34±0.30^{ab}	26.79±0.15^{b}	39.28±0.45^{ab}	66.24±0.39^{ab}	91.73±0.68^{ab}	140.16±15.44^{ab}
Samples	**DPPH-scavenging activity**					**IC_{50}**
Pb	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
Hex	35.95±0.14^{ab}	41.36±0.14^{ab}	49.51±0.37^{ab}	56.04±0.78^{ab}	62.49±0.48^{ab}	91.51±5.70^{ab}
DCM	41.44±0.24^{ab}	46.84±0.14^{ab}	80.64±0.24^{ab}	95.89±0.48^{a}	99.84±0.14^{a}	12.59±1.30^{ab}
EA	20.01±0.28^{ab}	34.63±0.38^{ab}	64.44±0.38^{ab}	92.94±0.38^{ab}	99.09±0.28^{a}	26.47±1.71^{ab}
But	36.92±0.28^{ab}	39.34±0.14^{ab}	66.44±0.50^{ab}	89.35±0.00^{ab}	95.32±0.37^{ab}	22.08±2.31^{ab}
HE	32.82±0.14^{ab}	40.95±0.38^{ab}	87.02±0.62^{b}	89.24±0.28^{ab}	92.36±0.49^{ab}	15.06±1.28^{b}
Samples	**DPPH-scavenging activity**					**IC_{50}**
Ro	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
Hex	43.58±0.49^{ab}	48.19±0.36^{ab}	87.60±0.24^{ab}	93.32±0.41^{ab}	99.76±0.14^{a}	10.46±0.43^{ab}
DCM	42.63±0.28^{ab}	45.80±0.49^{ab}	71.71±0.14^{ab}	91.34±0.50^{b}	93.48±0.36^{b}	14.93±2.40^{b}
EA	42.31±0.24^{ab}	46.20±0.36^{ab}	90.46±0.24^{a}	91.82±0.28^{b}	93.56±0.17^{b}	11.46±0.55^{ab}
But	49.08±0.24^{ab}	51.00±0.36^{ab}	61.33±0.23^{ab}	77.86±0.27^{ab}	96.24±0.20^{ab}	1.59±0.21^{a}
HE	41.43±0.27^{ab}	47.16±0.28^{ab}	88.56±0.41^{ab}	90.62±0.14^{ab}	93.32±0.24^{b}	11.16±1.49^{ab}
Samples	**DPPH-scavenging activity**					**IC_{50}**
	1 (µg/ml)	10 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	**(µg/ml)**
BHT	18.50±0.65	25.90±0.64	86.00±0.56	91.40±0.28	94.02±0.51	16.36±1.63
AA	39.10±0.34	82.60±0.26	90.80±0.32	95.08±0.43	99.80±0.58	1.62±0.25

Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus and Ro: Rosmarinus officinalis.

Hex: hexane fraction, DCM: dichloromethane fraction, EA: ethyl acetate fraction, But: butanol fraction, HE: hydroethanol fraction, BHT: 2,6-di-tert-butyl-4-methylphenol and AA: ascorbic acid.

$^{a}P < 0.05$ compared with BHT, $^{b}P < 0.05$ compared with AA.

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Table 3. *Results of phytochemical analysis of the fractions of Lamiaceae species.*

Samples	**Phytochemical analysis**					
Mo	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Hex	+	+	-	-	+	-
DCM	+	-	-	-	+	-
EA	+	+	-	+	+	+
But	-	+	+	+	-	+
HE	+	+	+	+	-	+
Me	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Hex	-	+	-	-	+	-
DCM	-	+	-	-	+	-
EA	-	-	+	-	+	+
But	-	+	-	-	+	+
HE	-	+	-	+	-	+
Ob	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Hex	+	+	-	-	+	-
DCM	+	-	-	-	+	-
EA	-	+	+	+	-	+
But	-	+	-	+	-	+
HE	-	+	-	+	-	+
Pb	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Hex	+	+	-	-	+	-
DCM	-	+	-	-	+	+
EA	-	+	-	+	+	+
But	-	+	+	-	+	-
HE	+	+	+	-	-	+
Ro	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Hex	-	+	-	-	+	-
DCM	-	+	-	-	+	-
EA	-	+	+	+	+	+
But	-	+	-	+	-	+
HE	-	-	+	-	-	+

Mo: Melissa officinalis, Me: Mentha sp., Ob: Ocimum basilicum, Pb: Plectranthus barbatus and Ro: Rosmarinus officinalis.

Hex: hexane fraction, DCM: dichloromethane fraction, EA: ethyl acetate fraction, But: butanol fraction and HE: hydroethanol fraction.

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