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Composition, Total Phenolic Content and Antioxidant Activity of the Essential Oil of Four Lamiaceae Herbs

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ABSTRACT

The composition of the essential oils of fresh aerial parts of marjoram (*Majorana hortensis*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata* L.) and rosemary (*Rosmarinus officinalis* L.) herbs were determined by GC-MS. The main identified oils constituents were γ-terpinene (19.77%), sabinene hydrate (17.56%), terpinen-4-ol (14.96%), α-terpinene (13.25%) and sabinene (12.35%) in *M. hortensis*; menthone (36.58%) and neo-menthol (40.47%) in *M. piperita*; carvone (42.84%) and carveol (34.98%) in *M. spicata* and 1,8-cineol (21.55%), α-pinene (17.77%) and camphor (15.38%) in *R. officinalis*. The 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity of the oils while the Folin–Ciocalteu method was used to determine the total phenolic equivalent. Peppermint oil has the highest free radical scavenging activity (IC_{50} = 59.19 μg mL^{-1}) and the most total phenolics. The lowest radical scavenging activity was exhibited by marjoram oil (IC_{50} = 65.352 μg mL^{-1}). Moreover, the radical scavenging activity of the four essential oils was much lower than that observed for the synthetic antioxidant TBHQ (IC_{50} = 29.81 μg mL^{-1}). The four Lamiaceae oils can be potential sources of natural antioxidant agents in particular, peppermint and rosemary oils, which have the highest total phenolic equivalent (0.163, 0.128 mg of gallic acid equivalents per 100 μl essential oil, respectively).

Keywords: GC-MS, *Majorana hortensis*, *Mentha piperita*, *Mentha spicata*, radical-scavenging activity, *Rosmarinus officinalis*
Abbreviations: BHA, butylated hydroxyanisole; **BHT**, butylated hydroxytoluene; **DPPH**, 2,2′-diphenyl-1-picrylhydrazyl; **EO**, essential oil; **GAE**, gallic acid equivalent; **GC–MS**, gas chromatography-mass spectrometry; **PG**, propyl galate; **TBHQ**, tertiary butyl hydroquinone

INTRODUCTION

Marjoram (*Majorana hortensis*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata* L.) and rosemary (*Rosmarinus officinalis* L.) are among the most important members of the Lamiaceae family. Marjoram is used worldwide as a spice and crude drug and possesses high antioxidant and anticancer properties (Rameilah 2009). *Mentha* is a genus of widely distributed aromatic perennial herbs with considerable economic importance and whose aerial parts are often used as a condiment (Bhat *et al*. 2002). The essential oils (EOs) of peppermint and spearmint are processed into flavoring for food, medicine, mouthwash and confectionery (Chambers and Hummer 1994). Peppermint EO has anticancer activity (Kumar *et al*. 2004). On the other hand, rosemary is one of the most effective spices widely used in food processing, is well cultivated in Egypt, and is the only spice commercially available for use as an antioxidant in Europe and the United States (Yanishlieva *et al*. 2006). The antioxidant properties of rosemary are well documented (Okoh *et al*. 2011; Kadri *et al*. 2011). Rosemary extract may be a good candidate for functional foods as well as for pharmaceutical plant-based products (Moreno *et al*. 2006).

The chemical composition of the EOs of Lamiaceae species is very variable. The major components were found to be terpinen-4-ol, gamma-terpinene, α-terpineol, sabinene and *trans*-sabinene hydrate in marjoram (El-Ghorab *et al*. 2004; Verma *et al*. 2010a, 2010b) in addition to β-caryophyllene, terpinolene, absence of sabinene hydrate and presence of new compound viridiflorene (Alarmal mangai and Ravi 2012); menthone, menthol, isomenthone, 1,8-cineol and neo-menthol in peppermint (Gupta and Saxena 2010; Yang *et al*. 2010; Mkolo *et al*. 2011); carvone, carveol and limonene in *M. spicata* (El-Keltawi and Croteau 1986; 1987; Hussaina *et al*. 2010; Mkolo *et al*. 2011) and 1,8-cineol, camphor, α-pinene as well as borneol in rosemary (El-Massry *et al*. 2008; Yang *et al*. 2010; Minaiyan *et al*. 2011). The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest (Aberoumand 2011; Hussain *et al*. 2011) since the most widely used synthetic antioxidants in food, namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butyl hydroquinone (TBHQ) have been suspected of causing or promoting negative health effects (Pokorny 1991; Suhaj 2006).

Moreover, due to the carcinogenic potential of synthetic antioxidants, natural phenolic antioxidants are being promoted as food preservatives and diet supplements (Shetty 1997; Botsoglou *et al*. 2002). As a natural source of antioxidants, wild herbs, spices, fruits, nuts, leafy vegetables and EOs from aromatic plants have been studied, for example, those of oregano (Kulisic *et al*. 2004) and rosemary (El-Massry *et al*. 2008). Using three different assay systems, limonene in celery seed, α-pinene in juniper berry, myristicin in parsley seed and germacrene in ylang−ylang showed high antioxidant activity (Wei and Shibamoto 2007). Eugenol, carvacrol, and thymol also possess the potential as natural agents for food preservation (Juliani and Simon 2002; Lee *et al*. 2005).

A literature survey indicated that the identification of terpenoids, antioxidant activity and radical scavenging activity of EOs and extracts of some *Lamiaceae* species were studied by others. Among the many studies to determine the antioxidant activities of marjoram (*Majorana hortensis*), peppermint (*Mentha piperita*), spearmint (*Mentha*

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spicata L.) and rosemary (*Rosmarinus officinalis* L.), most studies have focused mainly on the antioxidant activities of crude extracts. Although these four culinary and medicinal fresh herbs are mainly used for their distinctive aromas, there have been few studies on the identification of aroma components of the four distilled fresh herbs cultivated in Egypt. Our study was performed to evaluate the constituent make-up of EOs extracted from these herbs, as well as their total phenolic equivalent and antioxidant ability. Such results on the chemical composition of the EOs would allow for the identification of the best potential source of natural antioxidants.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves, stems and flowers (in the case of marjoram only)) of the four Lamiaceae species were collected from the Ancient Modern Organic Farm (AMOF), 10 Km from Alamaen off the Alexandria-Cairo desert road, Egypt in April 2010 during the flowering season.

EO isolation method

Quantitative determination of EO from fresh and air-dried samples of all four herbs was achieved by hydro-distillation for 3 h using a Clevenger-type apparatus. Oil yield per plant on a fresh and air-dried weight basis were determined. The yield of EO produced per plant was calculated by multiplying the average of fresh or dry herb weight of the plant by the average oil percentage. The obtained oil was dried over anhydrous sodium sulphate and after filtration, stored in a sealed vial at -4°C until tested and analyzed.

Gas chromatography-mass spectrometry (GC–MS) analysis

The EOs of the four fresh herbs were analyzed by GC/MS at the National Research Centre, Dokki, Cairo, Egypt using a Varian 3400 GC equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm; i.d. 0.25 µm film thickness). The multi-step temperature program was increased from 60°C (held for 3 min) to 260°C (held for 10 min) at a rate of 5°C min^{-1}. The carrier gas was helium at a flow rate of 1 ml min^{-1} and the sample size was 1 µl (injector temperature was 250°C). The mass spectrometer was a Varian-Finnigan SSQ 7000 operating with an ionization voltage of 70 eV. Scan time and mass range were 5 s and 40-400 m/z, respectively.

Compound identification

Identification of EO constituents was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra. Retention index was calculated for each compound using the retention times of a homologous series of C_6 - C_{26} *n*-alkanes (Adams 2001).

DPPH radical scavenging assay

The antioxidant activity of the four studied EOs was assessed on the basis of the scavenging activity of the stable 2,2′-diphenyl-1-picrylhydrazyl free radical (DPPH; Sigma Chemical Co.; St Louis, MO, USA) according to Miliauskas *et al*. (2004). Various concentrations of all four EOs (i.e., 25, 50, 100 and 200 µg/ml) were diluted five times with DPPH solution in methanol. The blank consisted of a 0.4 mM methanolic solution of DPPH. After 30 min incubation at room temperature, the reduction in the number of free radicals was measured by reading the absorbance at 517 nm using a Jenway 6405 UV-Vis spectrophotometer. TBHQ (Sigma) was used as the reference standard. All determinations were performed in triplicate. The percentage inhibition of DPPH radical by each EO was calculated according to the following formula (Yen and Duh 1994):

$$\% \text{ Inhibition} = [(A_B - A_A)/ A_B] \times 100$$

where A_B absorption of blank sample (t = 0 min) and A_A = absorption of tested oil (t = 30 min).

IC_{50} values, which represented the concentration of EO or TBHQ that caused 50% scavenging, were determined from the plot of inhibition percentage against concentration.

Determination of total phenolic compounds

The total phenolic equivalent of the four studied EOs was determined according to the method described by Taga *et al*. (1984). Briefly, 100 µl of each pure (100%) EO was dissolved in 10 ml of methanol, and 2 ml of this solution was made up with 0.3% HCl to 5 ml. A 100-µl aliquot of the resulting solution was added to 2 ml of 2% Na_2CO_3 and after 2 min, 100 µl of Folin-Ciocalteau reagent (Merck, Darmstadt, Germany) (diluted with methanol 1:1) was added and mixed well. After 30 min incubation, the absorbance of mixtures was recorded spectrophotometrically at 750 nm. The total phenolic contents were calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of gallic acid per 100 µl of EO sample.

Statistical analysis

Hydro-distillation of EOs, determinations of fresh and dry weight of herbs, antioxidant activity and total phenolic contents were conducted in triplicate. Data were expressed as mean ± standard deviation. Analysis of data was carried out according to Snedecor and Cochran (1990), and means were compared using least significant difference (LSD at the 5% level).

RESULTS

Growth parameters

Table 1 indicates the mean values of the growth parameters of marjoram peppermint, spearmint and rosemary. Marjoram recorded the highest fresh and dry matter. The mean values of the growth of spearmint were significantly higher than those of peppermint which could be attributed to the fact that more height and branching increased new shoots which resulted in a higher accumulation of fresh and dry matter.

EO yield and composition

Distillation of the fresh and air-dried aerial parts of marjoram, peppermint, spearmint and rosemary yielded (in %) (0.592, 1.625), (0.403, 1.213), (0.314, 0.756) and (0.375, 0.802), respectively, and in all cases, a pure light colourless oil. Oil yield per plant on a fresh and air-dried weight basis (ml EO/plant g) of marjoram, peppermint, spearmint and rosemary plant were (0.390, 358), (0.168, 0.155), (0.145, 0.122), (0.146, 0.142), respectively (**Table 2**). Marjoram recorded the highest mean value of EO percentage and yield per plant on a fresh and air-dried weight basis. In contrast, spearmint recorded the lowest mean value of EO percentage and yield per plant on a fresh and air-dried weight basis.

The EOs obtained by hydrodistillation of four Lamiaceae herbs were analyzed by GC/MS (**Table 3**). In *M. hortensis*, 19 components were identified representing 98.89% of the total oil with γ-terpinene (19.77%), sabinene hydrate (17.56%), terpinen-4-ol (14.96%), α-terpinene (13.25%), sabinene (12.35%) and phellandrene (7.11%) as the main constituents followed by *p*-menth-1-en-8-ol (α-terpinol) (4.82%), α-pinene (2.07%) and α-myrcene (1.99%).

On the other hand, menthone (36.58%), neo-menthol (40.47%), 1,8-cineol (8.69%), menthol acetate (4.33%), sabinene (1.64%) and α-pinene (1.11%) were the main components among the 28 constituents characterized in the oil of *M. piperita* representing 99.67% of the total components detected. In *M. spicata*, 28 components were identified representing 99.54% of the total oil with carvone (42.84%), carveol (34.98%), limonene (4.28%), 1,8-cineol (2.12%), β-

Table 1 Growth parameters of marjoram, peppermint, spearmint and rosemary herbs cultivated in Egypt. Values are the means of three replicates ± S.D.

Lamiaceae species	Growth parameters			
	Plant height (cm)	No of branches/ plant	Fresh weight (g/plant)	Dry weight (g/plant)
Marjoram (*Majorana hortensis*)	38.57 ± 1.25	23.00 ± 1.00	65.89 ± 0.65	22.05 ± 0.62
Peppermint (*Mentha piperita*)	20.00 ± 1.0	17.33 ± 1.15	41.56 ± 1.05	12.78 ± 0.24
Spearmint (*Mentha spicata*)	35.33 ± 2.08	30.33 ± 2.08	46.28 ± 0.95	19.10 ± 1.08
Rosemary (*Rosmarinus officinalis*)	28.67 ± 1.53	17.00 ± 1.00	39.07 ± 1.10	17.77 ± 1.03
L.S.D. at 5%	3.06	2.60	1.60	1.86

Table 2 Essential oils contents; radical scavenging activity and total phenolic contents of tested essential oil of marjoram, peppermint, spearmint and rosemary herbs cultivated in Egypt. Values expressed are means of three replicates ± S.D.

Lamiaceae species	Essential oil content (ml EO/100 g)		Essential oil yield (ml EO/plant g)		Phenolic contents (mg /100 µl as gallic acid equivalent)	Radical scavenging activity IC_{50} value (µg/ml)
	FW	Air-dried	Fresh weight	Air-dried		
Marjoram (*Majorana hortensis*)	0.592 ± 0.021	1.625 ± 0.022	0.390 ± 0.004	0.358 ± 0.011	0.042 ± 0.006	65.35 ± 1.01
Peppermint (*Mentha piperita*)	0.403 ± 0.009	1.213 ± 0.084	0.168 ± 0.004	0.155 ± 0.013	0.163 ± 0.004	59.19 ± 0.65
Spearmint (*Mentha spicata*)	0.314 ± 0.002	0.756 ± 0.005	0.145 ± 0.003	0.122 ± 0.008	0.071 ± 0.010	63.80 ± 0.67
Rosemary (*Rosmarinus officinalis*)	0.375 ± 0.005	0.802 ± 0.002	0.146 ± 0.004	0.142 ± 0.009	0.128 ± 0.020	62.49 ± 0.66
L.S.D. at 5%	0.040	0.080	0.007	0.023	0.026	1.15

caryophyllene (2.03%), α-pinene (1.93%) and dihydrocarvone (1.79%) as the main constituents.

Moreover, the predominant compounds in the oil of *R. officinalis* were 1,8-cineol (21.55%), α-pinene (17.77%), camphor (15.38%), berbenone (8.46%), borneol (7.77%), linalool (5.06%), endoborneol acetate (3.84%), endoborneol (3.74%) camphene (3.53%), α-myrcene (1.72%) and β-caryophyllene (1.37%) among the 24 constituents characterized in the oil and representing 95.40% of the total components.

There was great variability in the chemical composition of EOs obtained from the four Egyptian aromatic plants. The presence of the monoterpene hydrocarbons α-pinene and α-myrcene, a high percentage of oxygenated monoterpenes (ranging from 37.34% in marjoram to 87.78% in peppermint) and the sesquiterpene hydrocarbon β-caryophyllene were the common factors for the four Lamiaceae EOs. On the other hand, a high concentration of 1,8-cineol characterized the peppermint and rosemary EOs (8.69 and 21.55%, respectively).

Total phenolic equivalent and antioxidant activity

Table 2 shows that variations in the radical scavenging activities and total phenolic equivalent of the four EOs investigated were statistically significant. The antioxidant activity of herbal EO was investigated using the DPPH assay. The IC_{50} values ranged from 59.19 to 65.35 µg mL^{-1}. Peppermint oil has the best free radical scavenging activity (IC_{50}= 59.19 µg mL^{-1}), followed by rosemary oil (IC_{50} = 62.49 µg mL^{-1}). The lowest radical scavenging activity was exhibited by marjoram oil (IC_{50} = 65.35 µg mL^{-1}). Moreover, the reducing power of the four EOs were much lower than that observed for the synthetic antioxidant TBHQ (IC_{50} = 29.81 µg mL^{-1}).

Total phenolic equivalent were determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalent in mg/100 µl. The total phenolic equivalent ranged from 0.042 to 0.163 mg/100 µl EO. The total phenolic equivalent among the four herbal EOs were as follows: peppermint > rosemary > spearmint > marjoram (**Table 2**). Overall, the highest and the lowest phenolic equivalent and antioxidant activity were found in peppermint and marjoram oils, respectively.

DISCUSSION

Essential oil composition

In the present study, the EO of four fresh herbs cultivated in Egypt was hydro-distillated to evaluate the EO yield, composition, antioxidant activity and total phenolic contents (**Table 3**). According to Alarmal Mangai and Ravi (2012), Osman *et al*. (2010), Atti-Santos *et al*. (2005), and El-Keltawi and Croteau (1987), the EO content on a fresh weight basis of marjoram (South India), peppermint (Sudan), rosemary (Brazil) and spearmint (USA) were 0.25, 0.40, 0.37 and 0.50%, respectively. In Egypt, the EO content of cultivated marjoram was higher but peppermint and rosemary contained similar EOs, while spearmint contained less EO on a fresh weight basis. Our results may have differed due to different experimental conditions, which interfere with EO content and composition. A similar variation in the EO content on a fresh weight basis was Registered for *M. hortensis* cultivated in India, EO ranged from 0.7% (at flowering stage), 0.66% (at flower initiation) to 0.20% (early vegetative stage) (Verma *et al*. 2010b). Marotti *et al*. (1993) reported that EO yield and composition depend on pedoclimatic conditions and on the ontogenic stage of the plant. Previous research on the dried aerial parts of *Mentha piperita* (in Morocco) and *M. spicata* growing wild in Greece indicated a similar oil content to the Egyptian counterpart, recording 1.02% and ranging from 0.3 to 2.2%, respectively (Kokkini and Vokou 1989; Derwich *et al*. 2010), 0.6 to 0.8% in Turkey (Baser *et al*. 1999) and 0.8 to 1.9% in different accessions of Iranian *M. spicata* (Zeinali *et al*. 2005). The EO content of Egyptian *M. spicata* was similar to the EO content of wild plants growing in different parts of the world.

In the present work, although marjoram peppermint, spearmint and rosemary belong to the same family, EO synthesis and accumulation varied. Marjoram recorded the highest EO percentage and yield per plant on a fresh and air-dried weight basis due to the highest mean fresh and dry matter production. According to Czepak (1998), the higher the dry matter yield of plants the higher their EO yield. In contrast, spearmint had the lowest mean EO percentage and yield per plant on a fresh and air-dried weight basis, perhaps due to the small number of oil glands, reduced biosynthesis of monoterpenes and consequently low EO production. Moreover, EO yield per plant on a dry weight basis was lower than that on a fresh weight basis for the four studied herbs. Rabak (1917) suggested that a reduction in EO yield may occur if plants are dried before distillation, due to changes that favor the formation of esters and the production of free acids in *M. piperita*.

The major constituents of marjoram EO detected in this study (γ-terpinene, sabinene hydrate, terpinen-4-ol, α-terpinene, sabinene and phellandrene) were consistent with those of previously published studies but at different concentrations of individual components (Novak *et al*. 2002; Mishra *et al*. 2004; Verma *et al*. 2010a, 2010b). Variability in the volatile components among marjoram EO from our results appears to be largely due to stage of harvest, seasonal and environmental factors, in addition to the use of different methods of extracting the volatile components (El-

Table 3 Composition of essential oil of *Majorana hortensis*, *Mentha piperita*, *Mentha spicata* and *Rosmarinus officinalis* herbs.

Oil components (%)	*Rosmarinus officinalis*	*Mentha spicata*	*Mentha piperita*	*Majorana hortensis*	RI
Monoterpene hydrocarbons					
α-Pinene	17.77	1.93	1.11	2.07	939
Camphene	3.53	0.09	0.03	--	954
Sabinene	--	--	1.64	12.35	975
α-Myrcene	1.72	1.38	0.31	1.99	991
δ-3-Carene	1.52	0.04	--	--	1004
α-Phellandrene	--	--	--	7.11	1003
α-Terpinene	1.25	--	--	13.25	1018
Limonene	--	4.28	--	--	1031
γ-Terpinene	0.79	0.61	0.40	19.77	1062
Total	**26.58**	**8.33**	**3.49**	**56.54**	
Oxygenated monoterpenes					
1,8-Cineol	21.55	2.12	8.69	--	1030
Carveol	--	34.98	--	--	1229
Sabinene hydrate	--	1.19	1.03	17.56	1431
Limonene oxide	--	0.04	--	--	1139
Amyl isovalerate	--	--	0.16	--	1040
trans-Caran-4-ol	--	--	0.06	--	1118
Linalool	5.06	--	0.33	--	1085
Camphor	15.38	--	0.08	--	1122
p-Menthone	--	0.06	36.58	--	1154
neo-Menthol	--	--	40.47	--	1173
Dihydrocarvone	--	1.79	--	--	1193
Carvone	--	42.84	--	--	1242
Terpinen-4-ol (4-Terpinol)	--	--	--	14.96	1340
Endoborneol	3.74	--	--	--	1169
Borneol	7.77	--	--	--	1165
Berbenone	8.46	--	0.07	--	1194
Citronellol	0.28	--	--	--	1228
α-Terpineol (*p*-Menth-1-en -8-ol)	--	--	0.11	4.82	1295
Pulegone	--	--	0.20	--	1237
Total	**62.24**	**83.02**	**87.78**	**37.34**	
Sesquiterpene hydrocarbons					
α-Bourbonene	--	1.24	0.30	--	1384
α-Elemene	--	0.89	0.12	--	1391
β-Caryophyllene	1.37	2.03	1.49	1.92	1421
Aromadendrene	--	0.19	0.12	0.53	1439
α-Humulene	0.21	0.14	0.26	--	1454
δ-Cadinene	0.16	--	--	--	1524
δ-Muurolene	--	0.15	0.27	--	1477
Ledene	--	--	0.15	0.09	1533
α-Cadinene	0.33	0.21	0.85	0.02	1538
α-Cubebene	--	0.67	--	--	1351
Bicyclogermacrene	--	0.39	--	0.71	1494
Total	**2.07**	**5.91**	**3.56**	**3.27**	
Oxygenated sesquiterpenes					
Nerollidol	--	--	0.03	--	1534
Caryophyllene oxide	0.10	--	--	--	1581
Veridiflorol	0.04	--	0.46	--	1633
α-Cadinol	0.11	0.09	0.02	--	1653
Spathulenol	--	0.18	--	0.25	1576
α-Eudesmol	0.12	--	--	--	1652
Total	**0.37**	**0.27**	**0.51**	**0.25**	
Aliphatic esters					
3-Octanyl acetate	--	0.09	--	--	1215
Dihydrocarvyl acetate	--	1.56	--	--	1356
trans-Carvyl acetate	--	0.36	--	--	*1325*
Menthol acetate	--	--	4.33	0.02	1294
Linalyl actate	--	--	--	1.15	1257
Endobornyl actate	3.84	--	--	0.09	1259
Geranyl actate	0.21	--	--	0.23	1383
Total	**4.05**	**2.01**	**4.33**	**1.49**	
Aromatic compounds					
Methyl eugenol	0.09	--	--	--	1401
Unidentified compounds	4.60	0.46	0.33	1.11	

Keltawi and Croteau 1987; El-Ghorab *et al*. 2004). In India, considerable variations in the qualitative composition of the *Majorana hortensis* oils were obtained from different ages. The oil was mainly composed of monoterpenes and to a small extent sesquiterpenes. Oxygenated monterpenes (65.69-76.95%) dominated at late vegetative stage and flower initiation. On the other hand, monoterpene hydrocarbons increased and attained the maximum (15.08-29.65%) at flowering stage (Verma *et al*. 2010b).

In *M. piperita* EO, the main compound was neo-men-

thol followed by menthone, 1,8-cineol and menthol acetate. Similarly, neo-menthol was previously reported as major terpene in *M. piperita* (Gupta and Saxena 2010; Yang *et al*. 2010). El-Keltawi and Croteau (1986) showed percentages of chemical constituents in peppermint similar or close to our values although menthone was the major compound followed by menthol, isomenthone, 1,8-cineol and neo-menthol. In the present study, variability in the volatile components may be due to climatic, environmental factors and origin. In addition, *M. spicata* EO is rich in carvone and carveol, representing 77.82% of quantified total volatiles followed by limonene, 1,8 cineol, dihydrocarvone, carvyl acetate and sabinene hydrate. The essential oil profile of spearmints from this study was similar to EO from Pakistan (Hussaina *et al*. 2010). Maffei *et al*. (1986) previously reported carvone, dihydrocarvone and their related compounds carveol, carvyl acetate, dihydrocarveol, and dihydrocarvyl acetate as the main components in a number of spearmint EOs. Several authors (El-Keltawi and Croteau 1987; Zheljazkov *et al*. 2010; Mkolo *et al*. 2011) indicated the existence of carvone and limonene as the major components in *M. spicata*. Baser *et al*. (1999) reported the occurrence of menthone, isomenthone, *trans*-sabinene hydrate, carvone, terpinen-4-ol and 1,8-cineole, linalool and carvone-rich EOs in Turkish *M. spicata*. The dried aerial parts of 9 accessions of *M. spicata* L. could be divided into six different chemotypes with significant variation in EO composition between the accessions (Zeinali *et al*. 2005). The present study indicates that EO rich in carvone and carveol was distinctive for *M. spicata* cultivated in Egypt and Pakistan (Hussaina *et al*. 2010) which may be genetically different from *M. spicata* growing in other countries. Moreover, the analysis of *R. officinalis* EO revealed 1,8-cineol as the major compound followed by α-pinene, camphor and borneol, respectively. Previous reports showed chemical composition closer to our findings in the EO of fresh and dried *R. officinalis* leaves although the relative quantities of individual components varied; 1,8-cineole was the predominant compound (El-Massry *et al*. 2008; Yang *et al*. 2010) but in another study, α-pinene was the main compound followed by camphor and 1,8-cineol (Bernstein *et al*. 2009). Differences in the volatile components percent in our plant material might have been caused by climatic and seasonal factors, and the origin and stage of distillation.

Antioxidant activity

In the present study, peppermint and rosemary EOs, which contained a high amount of phenolic compounds (estimated as gallic acid mg/100 µl), also exhibited high antioxidant activity (determined by DPPH assay) (**Table 2**). The DPPH radical-scavenging assay proved that antioxidant activity of the rosemary EO (fresh aerial parts) in this study was higher than the antioxidant activity of rosemary EO (dried leaves) using a spectrophotometric or voltametric assay and scavenging effect on DPPH) radical using electron spin resonance (El-Massry *et al*. 2008; Kadri *et al*. 2011). However, by applying the DPPH assay, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (ABTS) and the ferric thiocyanate test, rosemary extract had a higher phenolic content and antioxidant activity than blackseed (*Nigella sativa* L.) EO (Erkan *et al*. 2008). The antioxidant activity of tailed pepper (*Piper cubeba* L.) EO was higher than that of the peppermint EO and TBHQ in our study (Singh *et al*. 2007). The antioxidant activity in decreasing order was cinnamon (extract) ≅ propyl gallate > mint (extract) (Murcia *et al*. 2004). According to Yang *et al*. (2010), lavender (*Lavandula angustifolia*) EO showed the highest free radical-scavenging capacity on DPPH, followed by peppermint and rosemary. On the contrary, the antioxidant activities of lavender EO was significantly lower than EOs of salvia (*Salvia officinalis*), lemon balm (*Melissa officinalis*), patchouli (*Pogostemon cablin*) and BHT (Hussain *et al*. 2011) and also lower than our four studied EOs and TBHQ. In the present study, scavenging abilities of TBHQ on DPPH radical was higher than that of jasmine EO, which in turn was higher than our peppermint EO. Also, the scavenging activity of our four studied Lamiaceae EOs was higher than that of angelica EO (Wei and Shibamoto 2007). Moreover, thyme (*Thymus vulgaris*) and oregano (*Origanum syriacum*) EOs presented better antioxidant profiles than *R. officinalis* and *M. hortensis* (Viuda-Martos *et al*. 2010) and the antioxidant activities of thyme and oregano EOs were higher than our four studied EOs and TBHQ. The DPPH radical scavenging activity of *O. syriacum* EO was nearly similar to that of the ethanolic extract of *O. heracleoticum* (Conforti *et al*. 2011) and higher than our *M. hortensis* EO (fresh aerial parts) and *O. vulgare* EO (air-dried flower tops and stalks) (Kulisic *et al*. 2004). In our study, when using the DPPH radical scavenging method, the antioxidant activity of the studied synthetic antioxidant (TBHQ) was higher than our four studied EOs, although nearly similar to that of *n*-propylgallate and BHA using the voltametric assay (El-Massry *et al*. 2008). Radical scavenging capacity on DPPH radical, chelating effect and hydroxyl radical scavenging effects supported that the antioxidant activity of both synthetic antioxidants BHA and BHT were relatively similar (Singh *et al*. 2007). Similarly, the antioxidant activities of salvia, lemon balm, patchouli and lavender EOs were significantly lower than that of BHT (Hussain *et al*. 2011), and that of tailed pepper EO was relatively lower than that of BHA and BHT (Wei and Shibamoto 2007). Also, oregano (*O. vulgare*) EO was less effective as an antioxidant than ascorbic acid, but comparable to α-tocopherol and BHT (Kulisica *et al*. 2004) (**Tables 4, 5**).

The antioxidant activities of EOs and other compounds from other studies (**Tables 4, 5**) (in relation to our results*) in decreasing order was: Tailed pepper ≅ thyme > oregano (*O. syriacum*) > *n*-propylgallate ≅ TBHQ* ≅ BHA ≅ BHT > jasmine > peppermint* > salvia > rosemary* > spearmint* > marjoram* > lemon balm > rosemary (dry aerial parts- southwest of Tunisia) > patchouli > lavender > oregano (*O. vulgare*) ≅ angelica. When observed from another perspective, i.e., the antioxidant activities of some Lamiaceae EOs (in relation to our results*), the decreasing order was: thyme > oregano (*O. syriacum*) > TBHQ* > peppermint* > salvia > rosemary* > spearmint* > marjoram* > lemon balm > rosemary (dry aerial parts- southwest of Tunisia) > patchouli > lavender > oregano (*O. vulgare*).

Tables 4 and **5** indicate that, as previously indicated (Koleva *et al*. 2002), that the antioxidant power depends on the chosen method, on the concentration and on the nature and physicochemical properties of studied antioxidants. The same antioxidant samples exhibit different antioxidative values depending on the concentration and the measured antioxidant parameter. Kulisic *et al*. (2004) concluded that the DPPH method is rapid, sensitive and requires small sample amounts. It is important to achieve a multiple different concentration measurements using different methods to avoid an incorrect conclusion.

In the present study, the highest antioxidant activity in peppermint and rosemary EO may be due to the considerable concentrations of α-pinene, menthol, 1,8-cineole, camphor and borneol. 1,8-Cineol, which was one of the major compounds present in the EO of peppermint and rosemary plants, highly inhibited hexanal oxidation (Lee *et al*. 2005). Similarly, high antioxidant activity has been attributed to the presence of α-pinene in juniper berry (*Citharexylum caudatum* L.), germacrene in ylang−ylang (*Cananga adorata*) (Wei and Shibamoto 2007). In addition, higher antioxidant activity due to phenolic content was also observed in *R. officinalis* extracts (Moreno *et al*. 2006).

Marjoram and spearmint EOs showed moderate phenolic compounds (estimated as gallic acid mg/100 µl) and antioxidant activity. The antioxidant activity was largely due to the presence γ-terpinene in the EO of marjoram in addition to carvone, carveol, 1,8-cineol and limonene in the EO of spearmint, and α-pinene in both EOs. Synergistic interactions among herbal EO components that had high antioxidant activity may have also had a role to play. Simi-

Table 4 Radical scavenging activity of essential oils, plant extracts and synthetic antioxidants using different bioassays methods.

Plant species	Test extract	Methods of determination	Radical scavenging activity	References and notes
1,8-Cineole 4-Terpinol	Authentic chemicals	Aldehyde/carboxylic acid assay (ACA)	% of hexanal Inhibitory effect 11 ± 0.9 at 50 µg/ml 14 ± 0.9 at 50 µg/ml	Lee *et al*. (2005)
Tailed pepper (*P. cubeba* L.)	EO, oleoresin (obtained using acetone as a solvent), reference standard (BHA, BHT)	1-Radical scavenging capacity on (DPPH) radical 2-Chelating effect 3-Hydroxyl radical scavenging effects	EO 71.2%, oleoresin 69.77%, BHA 96.41% and BHT 95.91% at 25 µl/ml	Singh *et al*. (2007) Three methods supported the antioxidant activity of EO and oleoresin. Both EO and oleoresin were relatively lower in comparison with synthetic antioxidants
Jasmine, angelica seed, parsley seed, rose, ylang−ylang	EOs	Scavenging abilities of the EOs on the DPPH radical	Ranged from 39% for angelica to 90% for jasmine at 200 µg/ml	Wei and Shibamoto (2007) Use of three different assay systems. The main compounds of EOs showing high antioxidant activity were limonene in celery seed, benzyl acetate in jasmine, α-pinene in juniper berry, myristicin in parsley seed, patchouli alcohol in patchouli, citronellol in rose and germacrene in ylang−ylang
Lavender (*L. angustifolia*), peppermint (*M. piperita*), rosemary (*R. officinalis*), lemon (*C. limon*), grapefruit (*C. paradisiaca*), frankincense (*B. carteri*)	Herb EOs	1) Free radical-scavenging capacity on the DPPH radical 2) Radical-scavenging activity against the ABTS radical 3) Lipid peroxidation in the linoleic acid system	The highest DPPH was obtained by the lavender EO Highest ABTS radical scavenging assay was obtained in peppermint EO Lavender oil was most effective for inhibiting linoleic acid peroxidation after 10 days.	Yang *et al*. (2010)
O. syriacum, *M. hortensis*, *R. officinalis*, *Cym. citratus*, *T. vulgaris*, *Art. annua*	Herb EOs	1) Free radical-scavenging capacity on the DPPH radical 2) % inhibition of TBARS 3) Metal chelating assay	Thyme 89.40% Inhibition of TBARS by oregano 85.79% Metal chelating, highly effective by marjoram, artemisia lemongrass	Viuda-Martos *et al*. (2010) Thyme and oregano EOs presented the best antioxidant profiles
Rosemary (*R. officinalis*)	EOs (leaves) solvent free microwave extractor (SFME), hydro-distillation (HD) methods	1) DPPH test 2) β-carotene bleaching test	1) SFME showed inhibitions of (48.80, 61.60 and 67.00%), HD oil (52.20, 55.00 and 65.30%) at 0.33, 0.5 and 1.00 mg/ml, respectively.	Okoh *et al*. (2011) The SFME extracted oil showed a higher activity than HD due to the higher proportions of oxygenated compounds in SFME extracted oils. The antioxidant property of Rosemary EO might be the combined activities of the various major and minor components of the oils.

ABTS = 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay; *Art.* = *Artemisia*; BCB = β-carotene bleaching test; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging method; *B.* = *Boswellia*; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; *C.* = *Citrus*; *Cym.* = *Cymbopogon*; EO = essential oil; *L.* = *Lavendula*; *M.* = *Majorana*; *M.* = *Mentha*; *O.* = *Origanum*; *P.* = *Piper*; *R.* = *Rosmarinus*; TBARS = thiobarbituric acid reactive species assay; *T.* = *Thymus*.

larly, limonene in celery seed, α-pinene in juniper berry, and 4-terpineol and carvone isolated from *Mentha spicata* showed high antioxidant activities (Lee *et al*. 2005; Elmastaş *et al*. 2006; Wei and Shibamoto 2007). The total oregano (*O. vulgare* L.) EO, the CHO fraction, pure thymol and carvacrol, as well as the hydrocarbon CH fraction exhibited almost the same antioxidant power (Kulisic *et al*. 2004). Ruberto and Baratta (2000) tested about 100 pure constituents of EOs and confirmed that the monoterpene hydrocarbons δ-terpinene, α-terpinene and *p*-cymene showed very high antioxidant activity. In our study γ-terpinene, α-terpinene and sabinene were the major components of the *M. hortensis* CH fraction. High antioxidant activity of sweet marjoram (*M. hortensis*) water extracts has also been reported (Triantaphyllou *et al*. 2001). The phenolic hydroxyl groups present in plant antioxidants have redox properties (Pietta 2000) allowing them to act as a reducing agent and a hydrogen donator in the DPPH assay. Thus, difference in composition of the herbal EO might result in their different antioxidant activity. Epidemiological studies have suggested a positive association between the consumption of phenolic-rich foods or beverages and the prevention of disease due to the presence of antioxidant components such as phenolics (Rice-Evans *et al*. 1997).

Free radicals in the human body have adverse effects on its immune system (Pourmorad *et al*. 2008). Antioxidants promote health and lower the risk of cancer, hypertension and heart disease (Valko *et al*. 2007) and protect the body from damage caused by free radical-induced oxidative stress (Souri *et al*. 2004). Rosemary (*R. officinalis* L.) and marjoram (*O. majorana*) extracts have potent natural antioxidant properties mostly due to their phenolic compounds (Hossain *et al*. 2008; Huda-Faujan *et al*. 2009), which has led to the use of rosemary, either in ground form or as an extract (Peng *et al*. 2005), and oregano (*O. vulgare* L.) EO in the food industry as a potential natural antioxidant additive (Kulisic *et al*. 2004).

CONCLUSIONS

EO synthesis and accumulation in four Egyptian aromatic plants was comparable to that of similar cultivated and wild growing plants in different parts of the world. Marjoram and spearmint had the highest and lowest mean EO percentage and yield per plant on a fresh and air-dried weight basis, respectively. The major constituents of marjoram, peppermint, spearmint and rosemary EOs detected in this study were consistent with those of previously published studies. EO rich in carvone and carveol was distinctive for *M. spicata* cultivated in Egypt. The DPPH radical scaven-

Table 5 Antioxidant activity of essential oils, plant extracts and synthetic antioxidants using different bioassays methods.

Plant species	Test extract	Methods of determination of antioxidant activity	IC_{50}	Reference and Notes
Oregano (*O. vulgare* L)	EO (air-dried flower tops and stalks) Reference standard BHT, α-tocopherol, ascorbic acid	1) DPPH radical scavenging method 2) β-carotene bleaching (BCB) test 3) Thiobarbituric acid reactive species (TBARS) assay	1) IC_{50} (g/l) EO 0.50 BHT 1.8×10^{-2} α-Tocopherol 8.6×10^{-3} Ascorbic acid 4.4×10^{-3}	Kulisic *et al*. (2004) The DPPH method is sensitive, requires small sample amounts and faster than BCB method. The same antioxidant samples exhibit different antioxidative values depending on the concentration and the measured antioxidant parameter.
Anise, cinnamon, ginger, licorice, mint, nutmeg, vanilla	Spice extracts Reference standard BHA, BHT propyl gallate	1-Trolox equivalent antioxidant capacity (TEAC) assay		Murcia *et al.* (2004) Capacity of antioxidant activity in decreasing order was cinnamon ≅ propyl gallate > mint > anise > BHA > licorice ≅ vanilla > ginger > nutmeg > BHT.
Rosemary (*R. officinalis*)	EO (air-dried leaves) Reference standard *n*-propylgallate, BHA	1) Spectrophotometric assay 2) Voltametric assay 3) Scavenging effect on DPPH radical using electron spin resonance (ESR)	1) IC_{50} (g/l) EO 0.250 n-propylgallate 0.0154 BHA 0.003 2) IC_{50} (g/l) EO 0.280 *n*-propylgallate 0.040 BHA 0.028	El-Massry *et al*. (2008) Rosemary EO has remarkable activity compared with the synthetic antioxidants at various concentrations. The IC_{50} values of both synthetic antioxidants are lower in comparison to that of rosemary volatile oil.
Rosemary (*R. officinalis*); blackseed (*Nigella sativa* L.)	Extract EO	1) DPPH assay 2) ABTS radical scavenging assay 3) Ferric thiocyanate test	Three test methods proved that rosemary extract had a higher antioxidant activity than blackseed EO.	Erkan *et al*. (2008) Rosemary extract have a higher phenolic content than blackseed EO. This fact explains the higher antioxidant activity of rosemary extract.
Oregano (*O. heracleoticum*)	Ethanolic extract of the aerial parts	1) DPPH test 2) β-carotene bleaching test	1) IC_{50} = 12.8 µg/ml 2) IC_{50} = 12.9 and 14.1 µg/ml at 30 and 60 min of incubation, respectively).	Conforti *et al*. (2011)
Lavender (*L. angustifolia*), patchouli (*Pogostemon cablin*), lemon balm (*Melissa officinalis*), salvia (*Salvia officinalis*)	EOs (aerial parts) Reference standard BHT	DPPH test	IC_{50} (µg/ml): Lavender 289.0, patchouli 225.7, lemon balm 69.9, salvia 62.3, BHT 9.9	Hussain *et al*. (2011) The EOs from *S. officinalis* and *M. officinalis* showed excellent radical scavenging activity, respectively. The antioxidant activities of *L. angustifolia* essential oil were significantly lower than other EOs and BHT.
Rosemary (*R. officinalis*	EOs (dry aerial parts) Reference standard BHT	1) DPPH test 2) β-carotene bleaching test 3) Reducing power antioxidant	1) IC_{50} (µg/ml): Rosemary 110.20, BHT 40.50 2) IC_{50} (µg/ml): Rosemary 27.28, BHT 20.00 3) EC_{50} (µg/ml): Rosemary 38.68, BHT 13.80	Kadri *et al*. (2011) Rosemary EO exhibited a slightly weak antioxidant potential than BHT
Marjoram (*M. hortensis*), peppermint (*M. piperita*), spearmint (*M. spicata*), rosemary (*R. officinalis*)	EO (fresh aerial parts) Reference standard TBHQ	DPPH radical scavenging method	IC_{50} (µg/ml): Marjoram 65.35, peppermint 59.19, spearmint 63.80, rosemary 62.49, TBHQ 29.81	Gharib and Teixeira da Silva (2012) In the present study, peppermint showed the highest antioxidant activity, followed by rosemary, spearmint and marjoram, respectively. IC_{50} value of TBHQ was lower in comparison to that of four EOs.

ABTS = 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay; *Art.* = *Artemisia*; BCB = β-carotene bleaching test; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging method; *B.* = *Boswellia*; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; *C.* = *Citrus*; *C.* = *Cymbopogon*; EO = essential oil; *L.* = *Lavendula*; *M.* = *Majorana*; *M.* = *Mentha*; *O.* = *Origanum*; *P.* = *Piper*; *R.* = *Rosmarinus*; TBARS = thiobarbituric acid reactive species assay; *T.* = *Thymus*., EC_{50} = concentration at which the absorbance is 0.5

ging assay proved that the antioxidant activity of four Egyptian aromatic plants in this study was higher than some studied Lamiaceae EOs namely lemon balm, patchouli, lavender (cultivated in Pakistan) and oregano (*O. vulgare*) EOs, although lower than thyme, *O. syriacum* EOs and a synthetic antioxidant, TBHQ. The antioxidant activity of the EOs from fresh aerial parts was higher than that of EOs derived from air-dried aerial parts. Overall, the highest and the lowest phenolic content and antioxidant activity were found in peppermint and marjoram oils, respectively. Peppermint EO exhibited high concentrations of menthol and 1,8-cineole which contributed to the antioxidant activity of its EO. Marjoram EO showed moderate antioxidant activity due to the presence of γ-terpinene. The four spices have natural antioxidant activity in the herbal EO and as such provide defense against cancer-inducing free radical damage. Furthermore, the inclusion of aroma compounds of the four spices in the diet should be part of any cancer preventive program. Peppermint, a member of the Lamiaceae, represents one of the best potential sources of potent natural antioxidants for the food industry as a natural antioxidant additive.

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