1

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Chemical Composition, Antioxidant and Antibacterial Activities of Lavender and Marjoram Essential Oils

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IN THIS STUDY we assessed the chemical composition, antioxidant and antibacterial activities of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) essential oils (EOs). Marjoram and lavender EOs showed promising antioxidant and free radical scavenging activities by 1,1-diphenyl-2-picryl-hydrasyl (DPPH) radical scavenging assay, ß-carotene bleaching test and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The three applied methods have shown that marjoram essential oil displays a strong antioxidant activity as well as good antibacterial activity.

GC/MS analyses indicated that *L. angustifolia* essential oil consisted of linalool as the most abundant component (37.65%), followed by linalyl acetate (15.29 %), α-muurolene (8.59 %), allo-aromadendrene (6.03%), ß-phellandrene (4.39%) and other minor components. On the other hand, *M. hortensis* essential oil consisted of terpinen-4-ol (48.98%), α-terpinol (11.75%), γ-terpinene (9.64%), spathulenol (3.51%), linalyl acetate (2.58 %) and sabinene hydrate (2.30%) as the main constituents.

Lavender and marjoram herbs presented considerable content of carbohydrates, protein, amino acids, crude fiber, EOs and minerals (Fe, Zn and Cu), low values of fats, ash and absence of mycotoxins.

Keywords: Lavender, Marjoram, Essential oil, Antioxidant, ABTS assay and Antibacterial.

Phytochemicals are sources of natural antioxidants used for health promotion, food preservation, food flavoring and cosmetics since they are safer to consumption and more environmentally friendly than their synthetic counterparts[1-4]. 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[1]. Spices and herbs are known for their health protective effect mostly attributed to their polyphenolic components, mainly flavonoids, phenolic acids, also, ascorbic acid and carotenoids which possess, antioxidant activity against the

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Reactive Oxygen Species (ROS)[5,6]. ROS induce oxidative damage to biomolecules like lipids, nucleic acids, proteins and carbohydrates. This damage causes the onset of many diseases such as rheumatoid, cirrhosis, arteriosclerosis, diabetes and cancer[7].

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease[8]. The antioxidant properties of many herbs and spices are reported to be effective in retarding the process of lipid peroxidation in oils and fatty acids and have gained interest of many research groups[9,10]. Among the herbs and spices extensively studied, the plants and EOs obtained from the Lamiaceae (Labiatae) family possess a significant antioxidant activity; for example, those of oregano[11,12] and lavender[4,13].

Also, numerous scientific reports have highlighted an important antimicrobial activity of essential oils[14,15], including mycotoxin-producing fungi, has also been tested[16]. These biological activities depend on the chemical compositions[17] which vary according to the geographical origin, environmental and agronomic conditions[18]. Therefore, the evaluation of the biological activity of an essential oil should be supplemented with the determination of its chemical composition[19].

Lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) are among the most important members of the Lamiaceae family. The Lavenders are a genus of about 25-30 species of flowering plants native to the Mediterranean region south to tropical Africa and to the many regions of Asia. The genus includes annuals, herbaceous plants, and small shrubs grow wild in the rocky soil[20]. Lavender has been used for centuries as an herbal remedy. It yields a highly effective essential oil with very sweet overtones and can be used in balms, salves, perfumes, cosmetics and topical applications. Internally, lavender essential oil is believed to be of benefit for a multitude of problems, including stress, headaches, colds, liver and gallbladder problems, loss of appetite, as mouthwash and all types of skin problems: wounds, psoriasis, insect bites, stings and as an insect repellent[21,22]. On the other hand, marjoram (*Majorana hortensis* L.), commonly known as 'sweet marjoram', is a perennial herb native to Cyprus and eastern Mediterranean countries[23]. Marjoram is used world wide as a spice and highly esteemed as condiment for seasoning food products. Essential oils (EOs) from aerial parts of the plants are used in the flavour, perfumery and pharmaceutical industries. Marjoram is well known for its medicinal and insecticidal values[24]. The plant is also reported to possess anticancer[25], antioxidant[12,26] and antimicrobial activities[27,28].

The chemical composition of the EOs of Lamiaceae species is very variable. The primary components of *Lavandula angustifolia* oil are linalool and linalyl acetate. Other components include α-pinene, limonene, 1,8-cineole, cis- and trans-ocimene, 3-octanone, camphor, caryophyllene, terpinen-4-ol, lavendulyl acetate and allo-aromadendrene[4,29-31]. In China, the most abundant component in

Lavender essential oil is 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate (43.73%), followed by 1,3,7-octatriene, 3,7-dimethyl- (25.10%), eucalyptol (7.32%) and camphor (3.79%)[32]. On the other hand, the major components of marjoram from different countries were found to be terpinen-4-ol, gamma-terpinene, α-terpineol, sabinene and trans-sabinene hydrate[26,33].

Aflatoxins and ochratoxim (mycotoxins) are secondary metabolites produced by *Aspergillus* and *Penicilliwn* genera[34,35]. They are among the most potent mutagenic and carcinogenic compounds known to be produced in nature[36]. The most important mycotoxin; aflatoxins are hepatotoxic, hepatocarcinogenic and ochratoxin, which is nephrotoxic and nephrocarcinogenic[37,38]. Potential contamination of the plants with mycotoxins is acutely and chronically toxic to both humans and animals. Several studies confined the occurrence of toxigenic mycoflora and mycotoxins in medicinal and herbal plants[38,39]. El-Kady *et al.*[40] reported the presence of aflatoxins (8–35 μg/kg) and sterigmatocystin (10–23 μg g/kg) in marjoram samples collected at Assuit Governorate, Egypt.

The aim of the present study was to examine the chemical composition, antioxidant (by using three different methods, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method; the ß-carotene linoleate model system and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay) and antibacterial activities of lavender and marjoram essential oils, also, to evaluate nutrient content as well as absence of mycotoxins (aflatoxins and ochratoxim) in both herbs for safer application in food preservation and in medicine.

Materials and Methods

Plant materials

Lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) were purchased from the Graduates Farm (GF), El- Minya, Governorate of the Western Assiut – Cairo desert road, Egypt in April 2010 during the flowering season. The herbs are free production of chemicals and solar dried (solar tunnel dryer).

Chemicals and reagents

All bio-chemicals employed in this study were of highest purity and obtained from E. Merck (Darmstadt, Germany) except, DPPH, TBHQ, ß-carotene, linoleic acid, aflatoxin and ochratoxin standards were purchased from Sigma Chemical Co. (Sigma–Aldrich Gmb H, Sternheim, Germany). All organic solvents were of AR grade.

Proximate composition determination

The proximate analysis is the time honored standard method of feed analysis. It involves simple chemical techniques used to distinguish nutrients from non-nutrients. Components of the proximate analysis (carbohydrate, fats, crude protein, moisture, crude fiber, nitrogen free extract and ash) were determined in lavender and marjoram samples according to the Association of Official Analytical Chemists (AOAC) methods[41,42]. Using weight difference, moisture

and ash were obtained. The fiber content was estimated from the loss in weight of crucible and its content on ignition. Carbohydrate was determined when the sum of the percentage of moisture, ash, crude protein and fats were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro- Kjeldahl method, involving digestion, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying with a factor of 6.25. The factor is derived from the generalization that most proteins contain 16% nitrogen.

Silica (acid insoluble ash) is not a component of the proximate analysis**.** Silica is determined by extracting an ash sample with hydrochloric acid to remove soluble minerals such as calcium, potassium and sodium salts. The material remaining consists mostly of silica.

Mineral contents, *i.e.* copper (Cu), iron (Fe) and zinc (Zn) were determined according to the method of AOAC[(43)] and Iva *et al.*[(44)], by inductive coupled plasma (ICP) "optima 2000".

Method for determining toxins

Extraction

Extraction of toxins from lavender and marjoram samples and the cleanup procedures were performed according to the AOAC[(45)]. Ground lavender and marjoram samples (50 g) were mixed with 2.5 g NaCl, 200ml of 80% (v/v) solution of methanol in water, 100 ml of *n*-hexane and blended in a blender for 5 min. 100ml of the slurry solution was then centrifuged to remove the fat content extracted into the upper phase. After filtration, 25ml of the lower phase was shaken for 1 min in 25ml of chloroform in a separatory funnel. Finally, the chloroform phase was separated and evaporated to dryness at 50°C. The extracted toxins in samples were then redissolved in 200 µL of methanol. Finally, 5µL of this solution was introduced into the injection port of the HPLC. Stock solutions of each mycotoxins were prepared by dissolving solid commercial toxin in the appropriate solvent at concentration of 1 mg/ml; aflatoxins in toluene/acetonitrile 99:1 and ochratoxins in toluene/acetic acid 99:1.

Identification

In order to verify the presence or absence of total alaflatoxins and ochratoxins in the lavender and marjoram samples, Immune affinity column and HPLC technique (Agillent 1200) series U.S.A were used_according to AOAC[(46)]. For aflatoxins determination column C_{18}, Lichrospher 100 RP-18, 5µm × 25cm were used. The mobile phase consisted of water: methanol: acetonitrite (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wave lengths for all aflatoxins were 362 and 460 nm (Flourences detector), respectively according to AOAC[(46)]. Ochratoxin were determined using column Nova- Pak C_{18} 4µm, 3.9 × 150mm. The mobile phase consisted of acetonitril: acetic acid : water (495 : 10 : 495 v/v/v) at flow rate of 0.8ml/min. The excitation and emission wave lengths for ochratoxins were 333 and 477 nm (Flourences detector), respectively according to AOAC[(46)].

Amino acids determination

Amino acids determination for both lavender and marjoram samples was performed according to the method of AOAC[47]. Sample of 20-30 mg weighted in conical flask and 5 ml of performic acid was added. The flask was closed and inserted in ice water bath for 16 hr. Sodium metabisulfate (1.0~1.5 g) and 25 ml of HCl (6 N) were added to the oxidizing mixture. The flask was then subjected to high temperature (110°C) for 24 hr in oven. The flask was then applied to concentration *in vacuo* till dryness using rota evaporator. A suitable volume of sodium citrate puffer (pH 2.20) was added to the dried film of hydrolyzed sample. The afforded soluble samples were then applied to the amino acids analyzer using Eppendorf LC 3000 (EZ Chrom, software used for data collection and processing). The results were calculated in percentage compared with the total crude protein.

Method for determining essential oils composition

Isolation

Quantitative determination of EOs from air-dried samples of lavender (*Lavandula angustifolia*) and marjoram (*majorana hortensis*) herbs was achieved by hydro-distillation for 3 hr using a Clevenger-type apparatus. The obtained oil was dried over anhydrous sodium sulphate and after filtration, stored in a sealed vial at -4°C until tested and analyzed.

GC-MS analysis

Quantitative determination of the main oil fractions of the two dry herbs was analyzed by GC/MS at Cairo University Research Park, Giza, Cairo. The GC analysis was carried out using HP 6890 Series/ GC system equipped with HP5973/Mass selective detector operating by electron ionization (EI) at 70 eV, and TR-FAME capillary column (30 m X 0.25 mm i.d, 0.25 µm film thickness). The multi step temperature program was increased from 80°C (held for 2 min) to 230°C (held for 2 min) with rate of 3°C min^{-1}. The carrier gas was helium at a flow rate of 2 ml min^{-1} and the sample size was 1 µl of diluted samples (5µl oil/2ml chloroform, v/v). Injector temperature was 250°C A spectral range of 35-500 *m/z* analysis was used. 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[48].

Antioxidant activity of lavender and marjoram essential oils

Diphenyl- 1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of the lavender and marjoram EOs was assessed on the basis of the scavenging activity of the stable 2,2′-diphenyl-1-picrylhydrazyl free radical according to Matthus[49]. Various concentrations of EOs (*i.e.,* 25, 50 and 100 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 on a Jenway 6405 UV-Vis spectrophotometer at 517 nm. TBHQ 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[50]:

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

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.

β-Carotene bleaching (BCB) method

Antioxidant activity of the lavender and marjoram essential oils was determined according to slightly modified version of the β-carotene-linoleate model system as described by Wettasinghe and Shahidi[51]. β-carotene in 0.2 ml of chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 (polyoxyethylene sorbitan monopalmitate) were mixed. After evaporation to dryness, under vacuum at 40°C, the resulting mixture was diluted with 10 ml of water and mixed well for 1 min to form emulsion A. Four milliliter aliquots mixtures were pipette into different test tubes containing 25, 50 and 100 µg/ml and TBHQ (the same concentrations in ethanol. TBHQ was used for comparative purposes. A control containing 0.2 ml of ethanol and 4 ml of the above emulsion was prepared. The tubes were placed in a water bath at 50°C and the readings of all samples were taken immediately (t=0) and at 15 min intervals until the colour of β-carotene disappeared in the control tubes (t =60 min) on a spectrophotometer at 470 nm. A mixture prepared as mentioned above without β-carotene served as blank. All determinations were performed in triplicate. The antioxidant activity (AA) of extracts was evaluated in terms of bleaching of the β-carotene using the following formula :

$$AA = 100[1 - (A_o - A_t)/(A^o_o - A^o_t)]$$

where : A_o and A^o_o are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A^o_t are the absorbance of test sample and control, respectively, after incubation for 60 min. The results were expressed in % basis in preventing bleaching of β-carotene.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

Antioxidant activity was measured using an improved ABTS method as described by Cai *et al.*[52]. The ABTS radical cation ($ABTS^+$) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23°C in the dark for 16 hr. The $ABTS^+$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^+$ solution (3.9 ml; absorbance of 0.700 ± 0.005) was added to 25, 50 and 100 µg/ml of the lavender and marjoram EOs and mixed vigorously. The reaction mixture was allowed to stand at 23°C for 6 min and the absorbance was immediately recorded at 734 nm. A standard curve was obtained by using Trolox standard solution at various concentrations

(ranging from 0 to 15 mM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox equivalents.

Microbial strains and antibacterial susceptibility test

Lavender and marjoram essential oils were individually tested against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichiea coli*, *Salmonella* and *Saccharomyces cerevisiae*. These species were supplied by the Regional Center for Food and Feed Safety Lab., Agriculture Research Center, Giza, Egypt. The broth dilution method described in the Manual of Clinical Microbiology(53) was used to assess the antibacterial activities of the lavender and marjoram EOs.

Minimal Inhibitory Concentration (MIC) test: Dilutions of each plant essential oil to be tested were prepared in 1.0 ml volumes of sterile brain heart infusion broth to give a range of concentrations. After preparation of suspensions of test microorganisms (ca.106 organisms per ml), one drop of suspension (0.02 ml) was added to the extract broth dilutions. After 24 hr incubation at 37°C, the tubes were then examined for growth. MIC was defined as the lowest concentration of essential oil, showing no visible bacterial growth after incubation time. Methanol was included in as negative control.

Statistical analysis

Hydro-distillation of EOs and determinations of antioxidant activities were conducted in triplicate. Data were expressed as mean ± standard deviation.

Results and Discussion

Phytoconstituents and quality control

As the vegetative parts of lavender with or without a small portion of the flowering tops of marjoram are used in the food and pharmaceutical industries, maintenance of quality of the produce is of utmost importance right from the harvesting stage onwards. All possible means should be taken to eliminate any chance of physical or microbial contamination.

Analysis of quality parameters of dry lavender and marjoram herbs includes fats (2.77, 1.49%), moisture (6.40, 7.70%), carbohydrates (40.53, 41.12%), proteins (11.65, 15.40%), fibers (25.36, 18.96%), ash (9.22, 11.45%), silica (4.07, 3.38%) and mineral elements such as iron (1767.0, 1608.0 ppm), zinc (39.84, 29.86 ppm), cupper (25.88, 14.94 ppm), respectively and absence of total aflatoxins and ochratoxin in both herbs (Table 1). Previous researchers on the typical chemical and physical specifications reported wide variations in the nutritional composition of Indian sweet marjoram(54). El-Kady *et al.*(40) highlighted the importance of quality control to prevent growth of fungi and bacteria in food products. They analyzed samples belonging to 24 kinds of marjoram spices collected from different places at Assuit Governorate (Egypt) for the natural occurrence of mycotoxins and reported for the first time the presence of aflatoxins (8–35 µg/kg) and sterigmatocystin (10–23 µg g/kg) in

different samples. However, they could not detect the presence of ochratoxin and zearalenone.

TABLE 1. Ingredients estimations of some chemical constituents of lavender (*L. angutifolia*) and marjoram (*M. hortensis*) dry herbs.

Ingredients (%)	**Lavender** (*l. angutifolia*)	**Marjoram** (*M. hortensis*)
Moisture	6.40	7. 70
Carbohydrates	40.53	41.12
Protein	11.65	15.40
Volatile oil	0.59	0.80
Fibers	25.36	18.96
Ash	9.22	11.45
Fats	2.77	1.49
Silica	4.07	3.88
Fe^{++} (PPm)	1767.0	1608.0
Zn^{++} (PPm)	39.84	29.86
Cu^{++} (PPm)	25.88	14.94

TABLE 2. Amino acids constituents of lavender (*L. angutifolia*) and marjoram (*M. hortensis*) herbs.

Amino Acids (%)	***L. angustifolia***	***M. hortensis***
Aspargine	0.76	0.83
Threonine*	0.34	0.36
Serine	0.35	0.41
Glutamine	0.85	1.120
Proline	0.38	0.43
Glycine	0.48	0.46
Alanine	0.46	0.68
Valine*	0.44	0.52
Isoleucine*	0.32	0.33
Leucine*	0.65	0.64
Tyrosine	0.00	0.34
phenylalanine*	0.12	0.46
Histidin*	0.12	0.16
Lysine*	0.26	0.38
Arginine*	0.24	0.80
Essential amino acids*	2.490	3.650
Non Essential amino acids	3.280	4.270
Essential amino acids*/ Non Essential amino acids	0.759	0.855

On the other hand, amino acids composition of lavender and marjoram plants (Table 2) indicates that the ratios of essential amino acids (threonine, valine, leucine, iso-leucine, phenylalanine, histidine, lysine and arginine) are less than non essential amino acids (aspargine, serine, glutamine, proline, glycine, alanine and tyrosine) representing 0.759 and 0.855 in lavender and marjoram plants, respectively. Glutamine, aspargine, glycine and alanine (from non essential amino acids), leucine and valine (from the essential amino acids) are the major amino acids in lavender and marjoram plants. In addition, proline and serine (from non essential amino acids), arginine and phenylalanine (from the essential amino acids) are the major amino acids in marjoram plants.

From our study, lavender and marjoram originated and processed in Graduates Farm (GF), El- Minya, Governorate of the Western Assiut–Cairo desert road, Egypt, may have a high reputation in the world market due to their high quality of amino acids, proteins, minerals, low value of fats, ash and absence of mycotoxins.

Essential oil

Essential oil yield

The presented data in Table 1 reveal that distillation of aerial parts of lavender and marjoram yielded 0.59% and 0.80% pure light colourless oil, respectively on air-dry weight basis. Previous research on the dried aerial parts of *Lavandula angustifolia* collected from the Botanical Garden, Faisalabad, Pakistan indicated similar oil content (0.58 g/100g) to the Egyptian counterpart[4]. On the other hand, according to Viuda-Martos, *et al.*[31], the EO yield was 1.4% for lavender (*L. officinalis*) collected from the city of Bilbeis in Sharkea region (NE, Cairo, Egypt) certified for organic biodynamic agriculture.

Moreover, Verma *et al.*[55], reported lower EO yield (0.35 % on fresh weight basis) of the *M. hortensis* collected from the lower region of Kumaon Himalaya, India than our yield (0.80%). Previous research on the hydro-distillation of aerial parts of *M. hortensis* cultivated in the Kumaon region of the western Himalaya, India registered variation in the essential oil content on fresh weight basis from 0.20 % (early vegetative stage); 0.32% (late vegetative stage); 0.66 % (flower initiation) to 0.70 % at flowering stage (33). Our results may have differed due to different experimental conditions and harvest crop stage, which interfere with EO content and composition. Marotti *et al.*[56] reported that EO yield and composition depend on pedoclimatic conditions and on the ontogenic stage of the plant.

Essential oil constituents

Table 3 shows the chemical constituents and their relative percentage of the total chromatogram area of lavender and marjoram EOs. GC/MS analyses of lavender EO identified 22 constituents; representing 97.53% of the total oil, linalool (37.65%) was found as the main component. linalyl acetate (15.29 %) was the second major component detected in lavender oil. α-Muurolene (8.59 %), allo-aromadendrene (6.03%) and ß-phellandrene (4.39%) other components identified in lavender oil. The profile obtained in this study was similar to that reported by Viuda-Martos, *et al.*[31], for lavender cultivated in Egypt they reported a higher value for linalool (39.83%),

linalyl acetate (32.11%), camphor (11.29%) and ß-phellandrene (7.63%). Hassiotis *et al.*[30], reported a similar oil component with our findings but at different concentrations in native plants of lavenda a new variety of *Lavandula angustifolia* cultivated in Greece and in Etherio were the main components linalool (20.1 and 26.9%, respectively) and linalyl acetate (13.3 and 22.8%, respectively). However, there was a great variability in chemical composition of lavender EO obtained in this study than lavender grown in China, 1,5-dimethyl-1-vinyl-4-hexenylbutyrate (43.73%) was the main constituent followed by 1,3,7-octatriene, 3,7-dimethyl- (25.10%), eucalyptol (7.32%) and camphor (3.79%)[32].

TABLE 3. Composition of essential oil of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) herbs.

Oil components (%)	RI	*L. angustifolia*	*M. hortensis*
α-pinene	939	-	1.35
β-pinene	981	2.52	-
β-phellandrene	1053	4.39	-
Sabinene hydrate	1073	-	2.30
γ-terpinene	1074	-	9.64
Linalool	1100	37.65	-
Camphor	1139	1.99	-
Borneol	1162	1.98	-
Terpinen-4-ol	1179	-	48.98
cis-p-menth-2-en-1-ol	1185	-	1.97
Piperitol	1194	-	0.97
α-terpineol	1195	-	11.75
Linalyl acetate	1261	15.29	2.58
Lavandulyl acetate	1283	2.39	-
Isoborneol acetate	1306	0.50	-
D-verbenone	1308	0.62	-
β-elemene	1393	1.15	-
γ-elemene	1425	-	1.76
β-farnesene	1445	4.35	
α-guaiene	1453	1.27	-
β-caryophyllene	1467	3.60	0.81
γ-muurolene	1475	0.58	-
β-guaiene	1483	0.90	-
Allo aromadendrene	1496	6.03	-
α-muurolene	1532	8.59	-
Nerolidol	1539	0.43	-
Caryophyllene oxide	1573	0.96	0.88
Spathulenol	1619	0.80	3.51
A-bisabolol	1662	0.56	
α-santalol	1672	0.98	-
Not identified compounds		2.47	13.50

In *M. hortensis*, 12 components were identified representing 86.50% of the total oil with terpinen-4-ol (48.98%), α-terpinol (11.75%), γ-terpinene (9.64%), spathulenol (3.51%), linalyl acetate (2.58 %) and sabinene hydrate (2.30%) as the main constituents followed by p-menth-1-en-2-ol (1.97%), γ-elemene (1.76%), α-pinene (1.35%), piperitol (0.97 %), caryophyllene oxide (0.88 %) and β-caryophyllene (0.81%). Similarly, the essential oil of *M. hortensis* cultivated in India was mainly composed of monoterpenes and to a small extent sesquiterpenes(55). *M. hortensis* oil from different countries possessed terpinen-4-ol as a major constituent representing 38.40% (Reunion Island), 30.55% (mid hills of north India), 24.25% (South India), 22.02% (subtropical India), 20.39% (Egypt) and 19.7% (Germany)(26,55,57-60). However, linalyl acetate (26.1%) followed by sabinene (12.0%) were the principle components of marjoram oil of Iran origin(61).

As mentioned above there was a great variability in the chemical composition of essential oils obtained from Egyptian lavender and marjoram herbs across provinces and countries. Such variability depends on several factors including local climatic and environmental conditions, season, geographical location, geology, genetic/chemotypic, nutritional status of the plants, part of the plant used and isolation process(32).

Antioxidant activity

Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method to establish authenticity(62). There are many different methods for determining antioxidant function each of which depends on a particular generator of free radicals, acting by different mechanisms(63). The DPPH method is sensitive, requires small sample amounts, faster than ß-Carotene-linoleic acid (BCB) assay and allows testing of both lipophilic and hydrophilic substances(64). On the other hand, the BCB method is helpful especially for investigations of lipophilic antioxidants and it is appropriate for the investigation of the antioxidant activity of essential oils(65). 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is applicable to both lipophilic and hydrophilic antioxidants, permits the measurement of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects(66). For this reason the antioxidant activity of lavender and marjoram essential oils was determined by these three complementary spectrophotometric methods.

DPPH Radical scavenging method

Many radical species of different reactivity are formed during a lipid oxidation. Relatively stable organic radical DPPH has been widely used in the determination of the antioxidant activity of single compounds as well as the different plant extracts(67). The DPPH assay measures the ability of the extract to donate hydrogen to the DPPH radical(68), resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity, which is reflected in a lower IC_{50}(69). Substrate polarity does not affect DPPH scavenging activity(65).

DPPH method was used to evaluate the antioxidant properties of the lavender and marjoram EOs in comparison with synthetic antioxidants TBHQ.

Table 4 shows a highly significant decrease in the DPPH radical concentration due to the scavenging activity of each oil concentration and standards. Marjoram EO showed a high radical scavenging activity (83.2%), followed by lavender EO (78.1%) and both EOs were less than positive controls TBHQ (89.7%) at 100 µg/ml. The values of IC_{50} was in the order TBHQ < Marjoram < Lavender. Similarly, using DPPH radical scavenging method, the antioxidant activities of Lavender (*L. angustifolia*) EO from aerial parts were significantly lower than patchouli (*Pogostemon cablin*); lemon balm (*Melissa officinalis*); salvia (*Salvia officinalis*) and reference standard BHT (IC_{50} = 289.0, 225.7, 69.9, 62.3 and 9.9 µg/ml, respectively[4] and lower than our lavender oil (IC_{50} =60.53). On the contrary, the highest DPPH was obtained by lavender EO; highest ABTS radical scavenging assay was obtained in peppermint (*M. piperita*) EO; lavender oil was most effective for inhibiting linoleic acid peroxidation after 10 days[13] . Kulisic *et al.*[11], found that IC_{50} = 0.50 and 1.8 × 10^{-2} (g/l) for EO (air-dried flower tops and stalks) of Oregano (*O. vulgare* L) and BHT, respectively and by the same method, ethanolic extract of the aerial parts Oregano (*O. heracleoticum*) gives IC_{50} = 12.8 µg/ml[70], which means higher antioxidant activities than our marjoram oil. Our results indicated different values for IC_{50} (µgmL^{-1}) for TBHQ by using DPPH and ß-Carotene, assays being 36.65 and 16.25, respectively. Similarly, IC_{50} (µgmL^{-1}) for BHT, using DPPH, ABTS, ß-carotene assays recorded 87.98, 77.85 and 36.85, respectively[71].

TABLE 4 . DPPH scavenging activity of different concentrations (25, 50, 100µG/Ml) of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) EOs and the synthetic antioxidants (TBHQ).

Lamiaceae species	**DPPH % inhibition**			
	25µg/ml	**50µg/ml**	**100µg/ml**	**IC_{50} (µg/ml)***
Lavender (*L. angustifolia*)	16.5±1.3	50.3±1.4	78.1±2.1	60.53±0.21
Marjoram (*M. hortensis*)	24.7±1.8	57.7±1.9	83.2±1.4	51.90±0.22
Synthetic antioxidants (TBHQ)	67.5±1.5	76.3±2.4	89.7±2.7	36.65±1.07

IC_{50} signifies concentration (µg/ml) for a 50% inhibition.

ß-Carotene-linoleic acid assay

ß-carotene bleaching test is based on the loss of the yellow colour of ß-carotene due to its reaction with radicals which are formed due to linoleic acid oxidation in an emulsion. The rate of ß-carotene bleaching can be slowed down in the presence of antioxidants. This fact is used in the antioxidant activity evaluation of the lavender and marjoram EOs in comparison with well known, synthetic TBHQ (Table 5).

TABLE 5. Antioxidant activity of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) EOs using the corresponding concentrations (25, 50, 100µG/Ml) measured by ß- carotene-linoleic acid method.

Lamiaceae species	β- carotene-linoleic acid method			
	25µg/ml	50µg/ml	100µg/ml	IC_{50} (µg/ml)
Lavender (*L. angustifolia*)	39.7±1.4	55.3±2.3	75.4±2.7	43.92±0.36
Marjoram (*M. hortensis*)	43.5±2.1	61.1±1.9	81.2±2.4	34.44±0.54
Synthetic antioxidants (TBHQ)	76.9±3.1	84.2±2.4	97.2±1.7	16.25±0.20

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The inhibition of TBHQ and EOs was concentration dependant. The antioxidant power decreased in the order TBHQ > Marjoram EO > Laveder EO. These data are consistent with the results obtained using DPPH assay. According to Viuda-Martos *et al.*[31], antioxidant activity of lavender (*Lavandula officinalis*)) EO and BHT measured by DPPH (IC_{50}= 48.7, 0.53 g/L) and inhibition of lipid peroxidation of buffered egg yolk (TBARS) assays (EC_{50} = 34.92, 0.001 g/L), where EC_{50} signifies concentration for a 50% inhibition. Other investigators previously reported that ascorbic acid, a well known polar antioxidant, showed a high antioxidant capacity by DPPH assay, but it was ineffective when tested by ß-carotene bleaching test. Indeed, the polar molecules remain in aqueous phase and are consequently less efficient in protecting linoleic acid[19,72]. The DPPH assay allows the test of both lipophilic and hydrophilic substances while ß-carotene bleaching test is dependent on substrate polarity. The high activity revealed by the ß-carotene test indicates a good ability to function at the lipid water interface. Our results revealed that lavender and marjoram EOs possesses a good capacity to scavenge free radicals and to prevent lipid peroxidation.

2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The method is based on the progressive consumption of antioxidant activity by the 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation ABTS(+) as it is generated in the reaction cuvette and can be automated spectrophotometricly at 734 nm in comparison with the antioxidant potency of standard amounts of Trolox, the water-soluble vitamin E analogue[73].

Table 6 shows the total antioxidant expressed as Trolox Equivalent Antioxidant Capacity, (TEAC) of lavender and marjoram EOs using the ABTS assay. The TEAC values ranged from 0.33 to 0.91 mM Trolox equivalent at 25-100 µg of *L. angustifolia* and *M. hortensis* EOs. However, *M. hortensis* essential oil showed greater (0.91 mM/100µg of oil) Trolox equivalent antioxidant capacity (TEAC) than *L. angustifolia* (0.83 mM/100µg of oil). The scavenging activity of the ABTS radical by lavender and marjoram EOs was found to be appreciable. An inhibitory effect of marjoram EO (IC_{50} = 36.18 ± 0.16 µgmL^{-1}) and lavender EO (IC_{50} = 42.60 ± 0.22 µgmL^{-1}) on ABTS free radicals was detected. This implies that the EOs may be useful for treating free radical-related

pathological damage (especially at a higher concentration). Studied lavender and marjoram EOs were less radical scavenging agents than TBHQ.

TABLE 6 . Antioxidant activity of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) EOs using the corresponding concentrations (25, 50, 100µG/Ml) measured by ABTS method.

Lamiaceae species	25µg/ml		50µg/ml		100µg/ml		IC_{50} (µg/ml)
	% inh	TEAC	% inh	TEAC	% inh	TEAC*	
Lavender (*L. angustifolia*)	39.1±1.3	0.33±0.01	55.7±1.7	0.52±0.02	81.7±2.0	0.83±0.02	42.60±0.22
Marjoram (*M. hortensis*)	41.4±2.1	0.35±0.01	61.4±2.4	0.59±0.02	88.6±1.9	0.91±0.03	36.18±0.16

* TEAC: Trolox equivalent antioxidant capacity (mM).

Moderate antioxidant activity in lavender and marjoram EOs can be ascribed to the considerable content of linalool, linalyl acetate, α-muurolene, allo-aromadendrene, β-phellandrene and β-pinene in the EO of lavender, in addition to terpinen-4-ol, α-terpinol, γ-terpinene, spathulenol, linalyl acetate, sabinene hydrate, piperitol, β-caryophyllene and α-pinene in the EO of marjoram. 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*), 4-terpineol in *Mentha spicata*[(74)] and phenolic content in rosemary (*Rosmarinus officinalis* L.) extracts[(75)]. Synergistic interactions among herbal EO components that had high antioxidant activity may have also had a role to play. Ruberto and Baratta[(76)] 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 was a major component of the *M. hortensis* CH fraction.

Antioxidants may act in various ways such as scavenging the radicals, decomposing the peroxides and chelating the metal ions[(77)]. All these activities may be related to the diverse compounds present in EOs, including phenolic, terpens and sesquiterpens. The action mechanism set in motion by the antioxidant activity of these compounds is still not clearly understood. Miguel[(78)] reported that these compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation by acting as chain-breaking peroxyl-radical scavengers. In addition, phenols directly scavenge reactive oxygen species. For Amensour *et al.* [(79)], the antioxidant activity of essential oil is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Thus, difference in composition of the EOs, specificity and sensitivity of each method might result in different antioxidant activity of the lavender and marjoram EOs by DPPH, ß-Carotene and ABTS assays and may be useful for treating free radical- related pathological damage.

Antibacterial activity

Table 7 summarizes the quantitative results (Minimal Inhibitory Concentrations, MIC) of the antibacterial effect of the essential oil from dry aerial parts of lavender and marjoram on the 6 reference strains assayed; two Gram negative bacteria "*Escherichiea coli and Salmonella*", three Gram positive bacteria "*Bacillus cereus, Bacillus subtilis and Staphylococcus aureus*" and yeast "*Saccharomyces cerevisiae*.

TABLE 7 . Antibacterial activity of lavender (*Lavandula angustifolia*) and marjoram (*Majorana hortensis*) essential oils on gram negative bacteria "*escherichiea coli* and *salmonella*"; gram positive bacteria "*Bacillus cereus, B. subtilis* and *Staphylococcus aureus*" and yeast "*Saccharomyces cerevisiae*".

Test microorganisms	MIC*	
	Lavender EO	**Marjoram EO**
Gram negative bacteria : *Escherichiea coli*	40 µl/ml	25 µl/ml
Salmonella	50 µl/ml	40 µl/ml
Gram positive bacteria : *Bacillus cereus*	10 µl/ml	5 µl/ml
Bacillus subtilis	40 µl/ml	15 µl/ml
Staphylococcus aureus	40 µl/ml	20 µl/ml
Yeast : *Saccharomyces cerevisiae*	40 µl/ml	20 µl/ml

Minimum Inhibition Concentration as % (v/v)

From the recorded minimum inhibition concentration (MIC) of the EOs, we observed that, the oil was active against all bacterial strains. However this activity varies between the test bacteria and MIC of the EOs ranged from 5 to 50 µl/mL (v/v). Interestingly, *E. coli* which is a cause of serious food poisoning and occasionally death was inhibited by marjoram and lavender oil at 25, 40 µl/ml, respectively. Similarly, the EO obtained from the aerial parts of *L. angustifolia* mill displays good antibacterial activity against four rhinitis-related bacteria including *E. coli*, *Staphylococcus aureus*, *Micrococcus ascoformans* and *Proteus vulgaris*. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the mostly likely reasons for its lethal action(32). In other studies, the bacteria demonstrating the biggest inhibition zones by diffusion method are not always the ones that present the lowest MIC and MBC (minimum bactericidal concentrations) values due to the fact that oil solubility and volatility affect the diameter of the growth inhibition zone(19). In our study, the recorded MICs showed that the Gram +ve strains of bacteria are more sensitive to marjoram and lavender oils than Gram -ve strains with the MICs (5-40 µl/ml). Marjoram EO exhibited the maximum activity against *Bacillus cereus* followed by *B. subtilis*.

Indeed, the majority of the essential oils assayed for their antibacterial properties showed a more pronounced effect against the Gram +ve bacteria[80]. The resistance of Gram -ve bacteria to essential oils has been ascribed to their hydrophilic outer membrane which can block the penetration of hydrophobic compounds into target cell membrane [81] .

In our study, the great antibacterial activity correlates with the high content of linalool (37.65 %), ß-pinene (2.52 %), low content of camphor and borneol in lavender oil and other components such as terpinen-4-ol, α-terpinol, γ-terpinene (48.98, 11.75 and 9.64 %, respectively) in marjoram oil. Several studies demonstrated that, the antibacterial activity of many essential oils and spices could be ascribed to the presence of phenolic components or substances other than phenolic compounds[82], presence of linalool and ß-pinene [83,48], oxygenated monoterpenes such as camphor, borneol, linalool and α-terpineol[85-87]. Moreover, the compounds present in the greatest proportions are not necessarily responsible for the total activity; the involvement of less abundant constituents should also be considered[88], the presence of synergy between the major components and other constituents of the oils leading to various degrees of antimicrobial activity. It has been reported that the strains of *E coli* that are not susceptible to the mixture of linalool-1,8-cineole are likely to be affected by linalool alone[89], which suggests that possible antagonistic and synergistic effects may occur according to the tested micro-organism[19].

Consequently, the antimicrobial activity of the investigated lavender and marjoram oils could be attributed to the high percentage of oxygenated monoterpenes and γ-terpinene the precursor of thymol (thymol inactivate bacterial strains in food system[90], synergistic effect of some compounds in the oils as well as oxygenated sesquiterpenes (α-santalol, caryophyllene oxide, spathulenol, α-bisabolol and nerolidol) should also be taken in consideration.

Conclusions

Egyptian lavender and marjoram herbs can be considered as good sources of natural compounds rich in nutrients, safer (due to absence of mycotoxin and fungal contaminants in both herbs) and may be effective substitutes for synthetically produced antioxidant and antimicrobial agents. Lavender and marjoram EOs could be safely used as a preservative material to control foodborne pathogens and spoilage bacteria, as a potential resource of natural antioxidants for food, cosmetic and pharmaceutical industries since their possible use as natural additives emerged from the tendency to replace synthetic preservatives with natural ones. Generally, marjoram was more effective than lavender in all studied trials. However, application of oils as a substitute for chemical fungicides, plant-origin products as antioxidant and antimicrobials needs to be addressed by regulatory authorities for most parts of these compounds

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التركيب الكيميائي للزيوت الطيارة لنباتات البردقوش واللافندر وتأثيرهما كمضادات للاكسدة والميكروبات

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تم دراسة التركيب الكيميائي للزيوت الطيارة لنباتي البردقوش واللافندر وتأثيرهما كمضادات للميكروبات بالاضافة الي تقديرنشاط مضادات الأكسدة للزيوت بأستخدام ثلاث أختبارات و هي: 2, 2́-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay) و طريقة (ß-carotene bleaching test) و طريقة (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) وتتلخص النتائج فى الآتى:

- سجل زيت البردقوش أعلي نشاط مضادات للأكسدة بالطرق الثلاثة المستخدمة وكذلك أعلي نشاط مضاد للبكتيريا مقارنة بزيت اللافندر.
- أظهر التحليل الكروموتوجرافي الغازي للزيوت الطيارة الناتجة من تقطيرالأجزاء الهوائية الجافة باستخدام جهاز GC-MS أن المكونات الرئيسية لزيت اللافندر هي: linalool (37.65%) يليها linalyl acetate (15.29 %) و α-muurolene (8.59 %) و allo-aromadendrene (6.03%) و ß-phellandrene (4.39%) بالاضافة لمكونات ثانوية أخرى.
- وأن المكونات الرئيسية لزيت البردقوش هي: terpinen-4-ol (48.98%) و α-terpinol (11.75%) و γ-terpinene (9.64%) و spathulenol (3.51%) و linalyl acetate (2.58 %). و sabinene hydrate (2.30%).
- تعتبر اعشاب اللافندر والبردقوش مصدر كبير للكربوهيدرات والبروتين والأحماض الأمينية و الألياف و الزيوت الطيارة والمعادن كالحديد و الزنك والنحاس و الدهون المنخفضة هذا بالاضافة الي خلوها من السموم الفطرية.