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Chemical Composition and Antioxidant and Anti-*Listeria* Activities of Essential Oils Obtained from Some Egyptian Plants

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The aim of this work was to (i) determine the chemical composition of the essential oils of six spices widely cultivated in Egypt (*Origanum syriacum*, *Majorana hortensis*, *Rosmarinus officinalis*, *Cymbopogon citratus*, *Thymus vulgaris*, and *Artemisia annua*); (ii) determine the antioxidant activity of the Egyptian essential oils by means of five different antioxidant tests; and (iii) determine the effectiveness of these essential oils on the inhibition of *Listeria innocua* CECT 910. There is a great variability in the chemical composition of essential oils obtained from the six Egyptian aromatic plants. Overall, thyme (highest percentage of inhibition of DPPH radical: 89.40%) and oregano (highest percentage of inhibition of TBARS: 85.79) essential oils presented the best antioxidant profiles, whereas marjoram, lemongrass, and artemisia were highly effective in metal chelating but had a pro-oxidative behavior by Rancimat induction test. Lemongrass essential oil showed the highest antibacterial activity against *L. innocua* with an inhibition zone of 49.00 mm, followed in effectiveness by thyme, marjoram, and oregano.

KEYWORDS: Essential oils; *Listeria innocua*; thyme; oregano; marjoram; lemongrass; artemisia; rosemary

INTRODUCTION

Many of the valuable sensory properties of food products diminish with time. This may be attributed to a large number of factors, including the actions of oxygen, light, and temperature that provoke the oxidation process. Oxidation of lipids may lead to a significant loss of a food's nutritional quality and the formation of off-flavors and toxic compounds (*1*). However, the most limiting factor of a food's shelf life is the growth of microorganisms, whether they are molds, bacteria, or yeasts (*2*). To avoid these problems, a wide variety of chemical preservatives are used throughout the food industry to prevent oxidation and the growth of spoiling and pathogenic microorganisms in foods (*3*). *Listeria innocua* is one of the six species belonging to the genus *Listeria* that are ubiquous (environment, soils, food sources, etc.) and may survive under extreme pH and temperature conditions and high salt contents (*4*). *L. innocua* is a nonpathogenic species closely linked to the presence of other *Listeria* species such as *Listeria monocytogenes*.

At present, there is increasing interest in the use of naturally occurring substances for the preservation of food. Aromatic and medicinal plants have also been the subject of study, particularly by the chemical, pharmaceutical, and food industries, because of their potential use in food for two principal reasons: (i) safety considerations regarding the potential harmful effects of the chronic consumption of synthetic compounds in foods and beverages; and (ii) consumer demand for the "elimination" of synthetic additives from foods, whereas "natural" additives are perceived as beneficial for both quality and safety aspects.

Recently, Li et al. (*5*) have reported that the use of antioxidant-rich species (cloves, cinnamon, oregano, rosemary, ginger, black pepper, paprika, and garlic) added to meat during cooking resulted in the reduction of meat oxidation and the in vivo reduction of plasma and urine malonaldehyde concentrations, providing scientific evidence of the health benefits of including antioxidant spices in rich fatty foods.

The essential oils (EOs) are a mixture of compounds present in aromatic and medicinal plants which, due to their content in phenolic compounds, terpenes, monoterpenes, and sesquiterpenes, are responsible for many antioxidant and antimicrobial properties. Both in vitro and in vivo studies have demonstrated how these substances act as antioxidants and show antibacterial activities (*6*, *7*). Li et al. (*5*) reported some biological effects of polyphenols including antioxidant activity, improvement of endothelial function, anti-inflammatory effect, and stimulation of DNA repair mechanism.

Aromatic plants and culinary herbs have long been the basis of traditional medicine in many countries. In Egypt, the growth of medicinal and aromatic plants presents a great socioeconomic interest as they are mainly produced for export. In this country, there are projects to recover the genetic diversity of these plants, as well as scientific research of the use, by traditional medicinal in Bedouin communities, of these plants. Indeed, there are a great number of rural jobs dependent on this sector. There are also many studies on the adaptation of foreign plants of medicinal

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interest to the Egyptian climate in order to scale up their production to create new jobs and opportunities for rural communities.

The aim of this work was to (i) determine the chemical composition of the EOs of six spices widely cultivated in Egypt [oregano (*Origanum syriacum*), marjoram (*Majorana hortensis*), rosemary (*Rosmarinus officinalis*), lemongrass (*Cymbopogon citratus*), thyme (*Thymus vulgaris*), and artemisia (*Artemisia annua*)]; (ii) determine the antioxidant activity of the Egyptian EOs by means of five different antioxidant tests; and (iii) determine the effectiveness of the Egyptian EOs on the inhibition of the growth of *L. innocua* CECT 910.

MATERIALS AND METHODS

Chemicals. Ascorbic acid, butylated hydroxytoluene (BHT), 2,2′-diphenyl-1-picrylhydrazyl (DPPH·), ferrozine, iron(III) chloride, iron(II) chloride, trichloroacetic acid (TCA), pentane, and Trolox were from Sigma Chemical Co. (Steinheim, Germany). Dibasic potassium phosphate, anhydrous sodium sulfate, 2-thiobarbituric acid (TBA), and dibasic sodium phosphate were from Merck (Darmstadt, Germany). Potassium ferricyanide was from Fluka BioChemika (Neu-Ulm, Germany). The solvent used for preparing standard solutions was methanol of HPLC grade, supplied by Merck.

Plant Materials. Oregano (*O. syriacum*), marjoram (*M. hortensis*), rosemary (*R. officinalis*), lemongrass (*C. citratus*), thyme (*T. vulgaris*), and artemisia (*A. annua*) were collected from the Sekem company plantation in the city of Bilbeis in the Sharkea region (northeastern Cairo) during the flowering period. The plantation is certified for organic biodynamic agriculture by COAE (Center of Organic Agriculture in Egypt).

Extraction of Essential Oil. The EOs of oregano, marjoram, rosemary, lemongrass, thyme, and artemisia were extracted from the entire plant (stems, leaves, and flowers) by hydrodistillation using a Clevenger-type apparatus for 3 h. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate (0.5 g). The extracted EOs was kept in sealed airtight glass vials and covered with aluminum foil at 4 °C until further analysis. The yields of the EOs were as follows: thyme, 1.6%; oregano, 0.6%; rosemary, 1.2%; lemongrass, 1.7%; marjoram, 0.4%; and artemisia, 0.8%

GC-MS Analytical Conditions. The volatile compounds were isolated, identified, and quantified on a Shimadzu GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GC-MS QP-5050A). The GC-MS system was equipped with a TRACSIL Meta X5 column (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 30 m × 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40 °C for 5 min; rising at 3.0 °C/min to 200 °C and held for 1 min; rising at 15 °C/min to 280 °C and held for 10 min. The injector and detector were held at 250 and 300 °C, respectively. Diluted samples (1:10 pentane, v/v) of 0.2 μL of the extracts were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 45−450. Most of the compounds were identified using two different analytical methods: (a) KI, Kovats indices in reference to *n*-alkanes (C_8−C_{32}) (*8*); and (b) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered to be tentative when it was based on mass spectral data only.

Antioxidant Activity. *2,2′-Diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Method.* The antioxidant activity of oregano, marjoram, rosemary, lemongrass, thyme, and artemisia EOs was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH (*9*). A volume of 50 μL of a methanolic stock solution of EOs of four different concentrations (50, 20, 10, and 5 g/L) was put into a cuvette, and 2 mL of 6×10^{-5} mol L^{-1} methanolic solution of DPPH was added. Ascorbic acid and BHT (in the same concentration) were used as reference. The mixtures were well shaken in a vortex (2500 rpm) for 1 min and then placed in a dark room. The decrease in absorbance at 517 nm was determined with a HP 8451 spectrophotometer (Hewell Packard, Cambridge, U.K.) after 1 h for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the radical without EO was used as control. The amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50% was calculated graphically. The inhibition percentage of the DPPH radical was calculated according to the formula

$$\%I = [(A_B - A_S)/A_B] \times 100$$

where I = DPPH inhibition %, A_B = absorbance of control sample (t = 0 h), and A_S = absorbance of a tested sample at the end of the reaction (t = 1 h). Each assay was carried out in triplicate.

Ferric Reducing Antioxidant Capacity (FRAC). The FRAC of the EOs was determined by using the potassium ferricyanide−ferric chloride method (*10*). One milliliter of different dilutions of EOs (50, 20, 10, and 5 g/L) was added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL of trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL of water and 0.5 mL of 1% $FeCl_3$. The absorbance at 700 nm was measured after the solution had been allowed to stand for 30 min. The FRAC of a sample is estimated in terms of Trolox equivalent antioxidant capacity (TEAC) in millimoles per liter Trolox. Each assay was carried out in triplicate.

Inhibition of Lipid Peroxidation of Buffered Egg Yolk by Essential Oils. The method of Daker et al. (*11*) was modified to determine the thiobarbituric acid reactive substance (TBARS), a secondary product of lipid peroxidation. For this, 0.1 mL of different dilutions of EOs (50, 20, 10, and 5 g/L) was added to a mixture that contained 1 mL of fowl egg yolk emulsified with 0.1 M phosphate buffer (pH 7.4), to obtain a final concentration of 25 g/L and 100 μL of 1 mM Fe^{2+}. The mixture was incubated at 37 °C for 1 h, after which it was treated with 0.5 mL of freshly prepared 15% TCA and 1 mL of 1% TBA. The reaction tubes were kept in a boiling water bath for 10 min. Upon cooling with ice, the tubes were centrifuged at 3500*g* for 10 min, to remove precipitated protein. The formation of TBARS was measured by removing 100 μL of supernatant and measuring the absorbance at 532 nm. The control was buffered egg with Fe^{2+} only. BHT and ascorbic acid were used as the standards. The percentage inhibition ratio was calculated from the equation

$$\%\ \text{inhibition} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control} refers to the absorbance of the control and A_{Sample} is the absorbance of the sample. To determine the concentration needed to achieve 50% inhibition of phospholipid oxidation in egg yolk, the percentage of lipid peroxidation inhibition was plotted against EO concentration. Each assay was carried out in triplicate.

Ferrous Ion Chelating (FIC) Ability. The FIC assay was carried out according to the method of Singh and Rajini (*12*) with some modifications. Solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted 20 times. Briefly, a solution (1 mL) of different concentrations of antioxidants (50, 20, 10, and 5 g/L) was mixed with 1 mL of $FeCl_2 \cdot 4H_2O$. After 5 min of incubation, the reaction was initiated by the addition of ferrozine (1 mL). The mixture was shaken vigorously, and after a further 10 min incubation period, the absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine−Fe^{2+} complex formation was calculated by using the formula

$$\text{chelating effect}\ (\%) = [(1 - A_S)/A_B] \times 100$$

where A_B = absorbance of control sample (the control contains $FeCl_2$ and ferrozine) and A_S = absorbance of a tested sample. Each assay was carried out in triplicate.

Determination of Oxidative Stability of Fat (RANCIMAT Assay). A Rancimat 743 (Methrohm, Switzerland) was used to determine the antioxidant lipid activity of oregano, thyme, rosemary, lemongrass, marjoram and artemisia EOs. The Rancimat worked on the following principle: A solution (100 μL) of different concentrations of different solutions of EOs (50, 20, 10, and 5 g/L) was added to lard (2.5 g), previously melted, giving final concentrations of 0.2, 0.08, 0.04, and 0.02% of antioxidant in the reacting system. The lard, with and without added antioxidant, was heated at 120 °C, and an air flow of 20 L/h was constantly blown into the mixture. The end of the induction period (IP) was characterized by the sudden increase of water conductivity, due to the dissociation of volatile carboxylic acids (*13*). The antioxidant activity index (AAI) was calculated from the measured induction times, according to the following formula:

$$\text{AAI} = (\text{induction period of lard with antioxidant}/\text{induction period of pure lard})$$

Table 1. Principal Constituents of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils and Their Relative Percentages of Total Chromatogram Area and Kovats Indices[a]

	Kovats index		% area					
compound	KI	Lit.[b]	*Thymus vulgaris*	*Rosmarinus officinalis*	*Majorana hortensis*	*Cymbopogon citratus*	*Origanum syriacum*	*Artemisia annua*
α-thujene	923	923	2.75		0.58		2.02	0.11
α-pinene	928	933	1.75	18.21	0.59		0.97	0.48
camphene	940	942	1.05	8.63				0.50
sabinene	963	961			5.02		4.91	0.63
myrcene	980	984	3.04	2.51	1.42	7.22	2.38	0.87
α-terpinene	1016	1018	3.1		5.23		7.41	
p-cymene	1024	1026	20.27	1.96	6.10	0.10	3.95	1.79
limonene	1028	1018	1.16		3.55		2.65	
1,8-cineole	1030	1031	1.46	23.59				8.13
γ-terpinene	1058	1059	21.19	0.49	9.26		18.96	0.43
artemisia ketone	1060	1062						13.96
α-terpinolene	1082	1082	0.19	0.57	2.36		1.75	
trans-β-ocimene	1097	1097			4.14		4.28	
linalool	1100	1100	2.60	1.67	3.32	0.87		
camphor	1137	1139	0.26	20.70	0.37			5.08
isoborneol	1156	1156		4.27				
terpinen-4-ol	1173	1178	1.34	1.73	41.43		17.20	1.10
α-terpineol	1188	1189		2.06	6.51		6.80	0.65
verbenone	1205	1204		4.05				
citral	1244	1240	0.11			37.44		
geranial	1278	1277	0.18			44.13		
thymol	1305	1304	32.23		0.64		21.04	
carvacrol	1309	1308	2.06				1.70	
trans-caryophyllene	1415	1418	1.59	0.12	1.44		0.96	7.73
β-farnesene	1450	1452						5.32

[a] Yields of essential oil extraction: thyme, 1.6%; rosemary, 1.2%, marjoram, 0.4%; lemongrass, 1.7%; oregano, 0.6%; artemisia, 0.8%. Compound identification was based on Kovats index and comparison with Wiley library. [b] NIST database.

An antioxidant activity index of >1 indicates inhibition of the lipid oxidation; the higher the value, the better the antioxidant activity. Each assay was carried out in triplicate.

Microbial Strains. The essentials oils were individually tested against *L. innocua* CECT 910. This species was supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia.

Agar Disk Diffusion Method. The agar disk diffusion method described by Tepe et al. (*14*) with some modifications was used to determine the antibacterial capacity of the EOs. Briefly, a suspension (0.1 mL of 10^6 CFU mL^{-1}) of *L. innocua* was spread on the solid medium plates (BHI agar; Sharlab, Sharlab SL, Barcelona, Spain). Sterile filter paper disks, 9 mm in diameter (Schlinder & Schuell, Dassel, Germany), were impregnated with 40 μL of the oil and placed on the inoculated plates; these plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

Determination of Concentration Effect. The concentration effect (CE) was studied to ascertain which doses of EO had an inhibitory effect on bacterial growth in the disk diffusion assay. The culture techniques used were those described in the previous paragraph (Agar Disk Diffusion Method), but adding 40, 20, 10, 5, and 2.5 μL of EO, which meant doses of 100, 50, 25, 12.5, and 6.25% of the initial volume (*15*). All tests were performed in triplicate.

Statistical Analysis. Conventional statistical methods were used to calculate means and standard deviations of three simultaneous assays carried out with the different methods. Statistical analysis (ANOVA) was applied to the data to determine differences ($p < 0.05$). To discover where there were significant differences between the levels of the main factor, contrasts (Tukey test) between means were made (*16*). For the antioxidant activity, ANOVAs with two factors (EO, oregano, thyme, lemongrass, rosemary, marjoram, artemisia, BHT, ascorbic acid; and concentration, 0.5, 1, 2, 5%) were applied for each parameter. For the antibacterial activity, ANOVA was made with the following factors: doses (five levels, 40, 20, 10, 5, and 2.5 μL) for each EO. The statistical analyses were made using Statgraphics 5.1 for Windows.

RESULTS AND DISCUSSION

Chemical Composition. The chemical composition of the EOs of *O. syriacum*, *M. hortensis*, *R. officinalis*, *C. citratus*, *T. vulgaris*, and *A. annua* was studied. The main constituents of each oil, their relative percentage of the total chromatogram area, and Kovats index are summarized in **Table 1**.

In the EO of thyme, 28 compounds were identified, representing 92.6% of the total oil, the major constituents being thymol (32.23%), γ-terpinene (21.19%), and *p*-cymene (20.27%). It is established that the terpenes thymol, *p*-cymene, and carvacrol are the major volatile components of thyme (*17*). Thymol and *p*-cymene were detected in Egyptian EO; however, carvacrol was present in low concentration (2.06%).

When the EO of rosemary was analyzed, 27 compounds were identified, representing 90.2% of the total oil, the major constituent being 1,8-cineole (23.59%). Other important compounds were camphor (20.70%) and α-pinene (18.21%). Tomei et al. (*18*) investigated the EOs from flowers and leaves of *R. officinalis* (collected from the wild in southern Spain) and found the main components to be camphor (32.33%), 1,8-cineole (14.41%), and α-pinene (11.56%), findings that are in agreement with the results presented here but at different concentrations. The geographical location of where the plant grows can also contribute to the content and quality of EO.

In marjoram EO 33 compounds were identified, representing 91.8% of the total oil, the main components being terpinen-4-ol (41.43%), γ-terpinene (9.26%), and α-terpineol (6.51%). The profile obtained in this study was very similar to that reported by El-Ghorab et al. (*19*) for *M. hortensis* cultivated in Egypt but with a lower value (20.39%) for terpinen-4-ol. In lemongrass only seven compounds were identified, representing 98.4% of the total oil, geranial (44.13%) and citral (37.44%) being the main constituents. It is established that these compounds are the main components of lemongrass EO (*20*). GC-MS analyses of oregano EOs identified 23 constituents; representing 94.5% of the total oil the main components were thymol (21.04%) and γ-terpinene (18.96%). In this case there are wide differences with most

Table 2. Antioxidant Activity of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils Measured by DPPH Method[a]

	DPPH % inhibition				
	5 g/L	10 g/L	20 g/L	50 g/L	IC_{50}[b]
Thymus vulgaris	55.47 ± 1.02 aA	70.03 ± 0.24 bA	71.04 ± 0.21 cA	89.40 ± 1.16 dA	4.50
Rosmarinus officinalis	4.30 ± 0.13 aB	6.53 ± 0.04 bB	11.28 ± 0.02 cB	21.88 ± 1.70 dB	121.61
Majorana hortensis	5.35 ± 0.16 aC	7.64 ± 0.11 bC	12.72 ± 2.25 cC	22.94 ± 1.18 dB	118.07
Cymbopogon citratus	4.00 ± 0.14 aD	5.82 ± 0.45 bD	7.93 ± 0.04 cD	14.80 ± 0.30 dC	199.63
Origanum syriacum	42.40 ± 1.24 aE	60.31 ± 0.02 bE	71.96 ± 0.15 cE	87.23 ± 1.62 dA	6.66
Artemisia annua	12.34 ± 0.09 aF	18.54 ± 3.53 bF	26.17 ± 0.36 cF	41.66 ± 0.24 dD	62.07
ascorbic acid	96.24 ± 1.16 aG	96.41 ± 2.15 aG	97.23 ± 0.76 aG	97.63 ± 1.25 aE	0.42
BHT	97.53 ± 1.36 aG	96.85 ± 3.13 aG	96.78 ± 0.96 aG	97.70 ± 1.41 aE	0.53

[a] Values followed by the same lower case letter within the same rwo are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. Values followed by the same upper case letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. [b] Concentration (g/L) for 50% inhibition.

Table 3. Antioxidant Activity of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils Measured by FRAC Method[a]

	TEAC[b] (mmol/L Trolox)			
	5 g/L	10 g/L	20 g/L	50 g/L
Thymus vulgaris	2.02 ± 0.00 aA	2.18 ± 0.02 bA	2.50 ± 0.01 cA	2.61 ± 0.00 dA
Rosmarinuss officinalis	0.52 ± 0.01 aB	0.71 ± 0.00 bB	0.82 ± 0.03 cB	0.81 ± 0.00 cB
Majorana hortensis	1.64 ± 0.07 aC	2.52 ± 0.00 bC	2.75 ± 0.01 cC	2.94 ± 0.01 dC
Cymbopogon citratus	0.71 ± 0.01 aD	1.60 ± 0.05 bD	2.76 ± 0.01 cC	2.95 ± 0.02 dC
Origanum syriacum	2.48 ± 0.06 aE	2.72 ± 0.02 bE	2.80 ± 0.00 cD	2.87 ± 0.01 dD
Artemisia annua	1.51 ± 0.05 aC	2.71 ± 0.02 bE	3.69 ± 0.05 cE	4.04 ± 0.04 dE
ascorbic acid	2.47 ± 0.03 aE	3.02 ± 0.01 bF	3.47 ± 0.01 cF	4.07 ± 0.02 dE
BHT	1.99 ± 0.01 aF	2.43 ± 0.02 bG	2.83 ± 0.02 cG	3.29 ± 0.01 dF

[a] Values followed by the same lower case letter within the same rwo are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. Values followed by the same upper case letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. [b] Trolox equivalent antioxidant capacity.

scientific papers about this plant (*21*, *22*), which report carvacrol and thymol as major components of *O. syriacum*. In artemisia EO 29 components were identified, representing 93.7% of the total oil, the main constituents being artemisia ketone (13.96) and 1,8-cineole (8.13%).

There is, then, great variability in the chemical composition of EOs obtained from Egyptian aromatic plants when compared with the same plant species from other locations. Such variability depends on several factors including climate, season, geographical location, geology, part of the plant, vegetative cycle, and the method used to obtain the EO (*23*).

Antioxidant Activity. There are many different methods for determining antioxidant function that rely on different generators of free radicals, acting by different mechanisms (*24*). It is very difficult to assess the antioxidant activity of a product on the basis of a single method. A single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail (*25*). Antioxidant activity assessment may require a combination of different methods because there are substantial differences in sample preparation, extraction of antioxidants (solvent, temperature, etc.), selection of end-points, and expression of results, even for the same method, so that comparison between the values reported by different laboratories can be quite difficult (*26*, *27*).

The radical-scavenging capacity of the spice EOs was tested using the "stable" free radical DPPH. **Table 2** shows the effective concentrations of each EO required to scavenge DPPH radical and the scavenging values as inhibition percentage. It can be seen that EOs exhibited various degrees of scavenging ability. Thyme EO showed the strongest ($p < 0.05$) radical-scavenging effect (89.40 ± 1.16) at 50 g/L, which is lower than those observed for the positive controls, BHT and ascorbic acid (97.70 ± 1.41 and 97.63 ± 1.25, respectively). This activity was followed by the oregano EO (87.23 ± 1.62) and artemisia EO (41.66 ± 0.24). Marjoram, rosemary, and lemongrass EOs showed the lowest scavenging activities ($p < 0.05$). The values of IC_{50} were in the order ascorbic acid < BHT < thyme < oregano < lemongrass < marjoram < rosemary < artemisia. The antioxidant activity measured by this method is slightly lower than that reported in the scientific literature for thyme, oregano, and rosemary EOs (*26*).

Table 3 shows the ferric reducing capacity obtained using the FRAC assay. A concentration-dependent ferric reducing capacity was found for all of the EOs studied. Artemisia EO, at the highest concentrations analyzed, showed the highest ($p < 0.05$) ferric reducing capacity in terms of Trolox concentrations with no statistically significant differences ($p > 0.05$) with positive control (ascorbic acid). It was followed by lemongrass, oregano, and marjoram with no statistically significant differences ($p > 0.05$) between them. Rosemary EO had little ferric reducing capacity compared with the other EOs.

Thyme, oregano, lemongrass, marjoram, rosemary, and artemisia EOs were examined for their ability to act as radical-scavenging agents and compared with the ability of ascorbic acid and BHT in the TBARs assay. Oregano EO showed ($p < 0.05$) the highest percentage of inhibition (85.79 ± 0.06) of all the EOs analyzed (**Table 4**), with no statistically significant differences ($p > 0.05$) with positive control (ascorbic acid). Artemisia and rosemary EOs showed the lowest percentage of inhibition values (44.55 ± 1.51 and 41.62 ± 0.34, respectively). All EOs studied were poorer radical-scavenging agents than ascorbic acid, whereas BHT showed the highest radical scavenging activity of all (90.22 ± 0.02). The EC_{50} values ranged from 0.001 to 59.38 mg mL^{-1}, and the lipid peroxidation inhibitory ability decreased in the order rosemary > artemisia > marjoram > thyme > oregano > ascorbic acid > BHT.

It should be noted that lemongrass EO showed a pro-oxidant activity. Recent work shows that EOs can act as pro-oxidants (*28*). This behavior may limit its potential use as an ingredient or preservative in food products.

Metals chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. Analysis of metal ion-chelating

Table 4. Antioxidant Activity of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils Measured by TBARS Assay[a]

	TBARS % inhibition				
	5 g/L	10 g/L	20 g/L	50 g/L	EC_{50}[b]
Thymus vulgaris	61.05 ± 0.27 aA	72.58 ± 1.15 bA	77.19 ± 0.57 cA	81.51 ± 0.57 dA	4.09
Rosmarinus officinalis	6.09 ± 0.86 aB	14.24 ± 0.48 bB	24.59 ± 4.35 cB	41.62 ± 0.34 dB	59.38
Majorana hortensis	34.82 ± 1.34 aC	61.27 ± 1.34 bC	69.46 ± 1.13 cC	76.23 ± 2.50 dC	6.27
Cymbopogon citratus					
Origanum syriacum	63.31 ± 1.05 aD	77.04 ± 1.51 bD	81.61 ± 0.71 cD	85.79 ± 0.36 dD	3.99
Artemisia annua	10.62 ± 1.24 aE	25.4 ± 1.41 bE	37.35 ± 0.80 cE	44.55 ± 1.51 dE	53.48
ascorbic acid	67.54 ± 3.57 aF	76.72 ± 0.10 bD	80.66 ± 0.17 cF	85.79 ± 0.06 dD	3.70
BHT	84.71 ± 0.06 aG	87.03 ± 0.10 bF	88.97 ± 0.25 cG	90.22 ± 0.02 dF	0.001

[a] Values followed by the same lower case letter within the same rwo are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. Values followed by the same upper case letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. [b] Concentration (g/L) for a 50% inhibition.

Table 5. Antioxidant Activity of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils Measured by Metal-Chelating Assay[a]

	FIC % inhibition				
	5 g/L	10 g/L	20 g/L	50 g/L	EC_{50}[b]
Thymus vulgaris	61.09 ± 0.57 aA	63.55 ± 0.18 bA	67.32 ± 0.63 cA	69.62 ± 0.45 dA	0.27
Rosmarinus officinalis	66.63 ± 0.03 aB	69.48 ± 1.74 bB	72.38 ± 0.23 cB	75.47 ± 0.10 dB	0.08
Majorana hortensis	81.20 ± 0.42 aC	84.29 ± 0.11 bC	86.04 ± 0.32 cC	88.02 ± 0.32 dC	0.001
Cymbopogon citratus	69.45 ± 0.69 aD	72.70 ± 0.29 bD	73.99 ± 0.22 cD	75.78 ± 0.62 dB	0.004
Origanum syriacum	64.11 ± 0.02 aE	71.99 ± 0.25 bE	78.11 ± 0.39 cE	85.46 ± 0.31 dD	0.89
Artemisia annua	62.25 ± 2.13 aF	68.87 ± 0.30 bF	79.04 ± 0.08 cF	98.03 ± 1.33 dE	2.73
ascorbic acid	21.59 ± 0.27 aG	27.77 ± 3.06 bG	35.55 ± 0.06 cG	38.54 ± 0.11 dF	79.51
BHT	29.75 ± 0.09 aH	32.87 ± 0.18 bH	35.54 ± 0.36 cG	37.08 ± 0.01 dG	138.42

[a] Values followed by the same lower case letter within the same rwo are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. Values followed by the same upper case letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. [b] Concentration (g/L) for a 50% chelating effect.

Table 6. Antioxidant Activity of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils Measured by Rancimat Method[a]

	antioxidant activity index (AAI)			
	5 g/L	10 g/L	20 g/L	50 g/L
Thymus vulgaris	1.05 ± 0.13 aA	1.20 ± 0.06 abA	1.29 ± 0.08 bA	1.53 ± 0.01 cA
Rosmarinus officinalis	1.01 ± 0.11 aA	1.04 ± 0.06 aB	1.02 ± 0.01 aB	0.96 ± 0.04 aB
Majorana hortensis	0.87 ± 0.01 aB	0.73 ± 0.10 bC	0.62 ± 0.04 bC	0.58 ± 0.10 bC
Cymbopogon citratus	0.76 ± 0.08 aC	0.68 ± 0.11 abC	0.45 ± 0.14 bC	0.23 ± 0.04 cD
Origanum syriacum	0.98 ± 0.12 aAB	0.99 ± 0.29 aABC	1.02 ± 0.01 aB	1.10 ± 0.08 aE
Artemisia annua	1.05 ± 0.02 aA	0.95 ± 0.05 bBC	0.96 ± 0.02 bD	0.71 ± 0.11 cC
ascorbic acid	1.02 ± 0.44 aABC	1.07 ± 0.13 aAB	1.13 ± 0.07 aE	1.44 ± 0.02 bF
BHT	1.23 ± 0.42 aABC	1.65 ± 0.04 aD	2.04 ± 0.65 bF	2.42 ± 0.23 bG

[a] Values followed by the same lower case letter within the same row are not significantly different ($p > 0.05$) according to Tukey's multiple-range test. Values followed by the same upper case letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple-range test.

properties showed that all of the EOs studied were capable of chelating iron(II) and did so in a concentration-dependent manner (**Table 5**). All of the EOs studied were better chelants of iron(II) than ascorbic acid and BHT. The highest concentration of artemisia EO showed the highest iron-chelating ability. At lower concentrations (5, 10, and 20 g/L) marjoram EO showed the highest values ($p < 0.05$) for chelating iron(II). Thyme EO at all concentrations showed the lowest values of metal-chelating ability. The main component of thyme EO is thymol, a monohydroxylated compound unable to form a complex with Fe^{2+}.

Marjoram and lemongrass EOs showed the lowest EC_{50} values (0.001 and 0.004 g/L, respectively). It should be noted that lemongrass EO showed lower metal-chelating activity than artemisia and oregano EOs and similar activity to rosemary EO. However, lemongrass showed better metal-chelating activity than these EOs on the basis of its lower EC_{50}.

Table 6 gives the Rancimat test values in terms of AAI of lard with the EOs added. The higher the induction period of the lard with the EOs added, compared with the control (pure lard), the better the antioxidant activity of that compound. The AAI, determined by the Rancimat method, decreased in the order BHT > thyme > ascorbic acid > oregano > rosemary. According to this method, only thyme, oregano, and rosemary EOs showed antioxidant activity, but lower than that of synthetic antioxidants (BHT, AAI = 2.42). Lemongrass, marjoram, and artemisia EOs showed pro-oxidant activity at all concentrations (values of AAI < 1).

Table 7 contains a summary of the antioxidant ability of EOs. Thyme and oregano showed a good antioxidant profile by all methods, followed by rosemary, which required higher doses. Marjoram, artemisia, and lemongrass failed as antioxidants and even acted as pro-oxidants, so limiting their applications in fatty foods as a function of oxidative risks. Antioxidants can be divided into two groups: preventive antioxidants and chain-breaking antioxidants. The first group comprises metal chelators. Free radical scavengers pertain to the second group. They scavenge free radicals and stop the propagation of free radical chain reactions (*25*).

The phenolic and terpenoid compounds present in the chemical composition of EOs are closely associated with their antioxidant function, mainly due to their redox properties exerted by various possible mechanisms: free radical scavenging activity, hydrogen

Table 7. Summary of Antioxidant Ability of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils As Assessed by Five Antioxidant Methods[a]

method	*Thymus vulgaris*	*Rosmarinus officinalis*	*Majorana hortensis*	*Cymbopogon citratus*	*Origanum syriacum*	*Artemisia annua*
DPPH	****	*	*	*	****	**
FRAC	***	*	***	***	***	****
TBARS	****	**	***	–	****	**
FIC	***	***	****	***	****	****
Rancimat	*	*	–	–	*	–

[a] –, pro-oxidant; *, low; **, low-medium; ***, medium-high; ****, high antioxidant activity.

donors, transition metal chelating activity, and/or singlet oxygen quenching capacity (*29*). However, it is very difficult to attribute the antioxidant effect of a total EO to one or a few active principles, because an EO always contains a mixture of different chemical compounds. In addition to the major compounds, also minor compounds may make a significant contribution to the oil's activity (*30*); it is even possible that the activity of the main components is modulated by other minor molecules (*31*).

As mentioned above, thyme and oregano EOs showed the highest antioxidant activity, measured by TBARS, DPPH, and Rancimat methods. The main component of these EOs is thymol (see **Table 1**). Considerable research (*32*, *33*) indicates the antioxidant activity of this compound through various methods such as free radical scavenging activity or hydrogen donors. Thymol, with greater steric hindrance of the phenolic group in comparison to carvacrol, had higher antioxidant activity. It is known that compounds with a hydroxyl group sterically hindered, such as BHT, possess a high antioxidative activity (*34*).

The activities of EOs such as antioxidants depend on several structural features of the molecules and are primarily attributed to the high reactivity of hydroxyl group substituents (*25*) but also to many other factors, such as concentration, temperature, light, type of substrate, and physical state of the system, as well as microcomponents acting as pro-oxidants or synergists that may influence the antioxidant activity (*35*).

Antibacterial Activity. The in vitro antibacterial activities of *O. syriacum*, *M. hortensis*, *R. officinalis*, *C. citratus*, *T. vulgaris*, and *A. annua* EOs against *L. innocua* CECT 910 were qualitatively and quantitatively assessed for the presence or absence of inhibition zones (**Table 8**).

The agar disk diffusion method indicated that lemongrass EO showed the highest antibacterial activity against *L. innocua* with an inhibition zone of 49.00 mm. The pro-oxidant effects of lemongrass EO can explain, at least in part, this antibacterial activity. Thus, Bakkali et al. (*36*) reported that pro-oxidant activities of volatile terpenic and phenolic components of EOs may damage eukaryotic cell membrane, in particular those of mitochondria, and thus promote the release of Ca^{2+}, cytochrome *c*, and ROS. This may lead to late apoptosis and/or necrosis including damage to proteins and DNA and overall cytotoxic effects (*37*).

The next most effective EO was thyme, which showed an inhibition zone of 40.00 mm. Oregano and marjoram EOs showed similar antibacterial activities, whereas artemisia EO was not active in the inhibition assay.

The CE on *L. innocua* inactivation can be seen in **Table 8**. The EOs of thyme, marjoram, lemongrass, and oregano,showed inhibitory effects ($p < 0.05$) at all added doses. The disks impregnated with 10, 5, and 2.5 μL of rosemary EO were not active against *L. innocua*, whereas artemisia EO had no inhibitory effects ($p > 0.05$) at any of the five doses tested.

With regard to thyme EO, significant differences ($p < 0.05$) were found between the 6.25, 12.5, and 25% doses; however, at higher doses (50 and 100%) no statistically significant differences were found ($p > 0.05$) on the inhibitory effect. The same was found in the case of lemongrass. For marjoram EO no statistically significant differences were found ($p > 0.05$) between the lower doses (6.25 and 12.5%). The highest dose (100%) showed significant differences ($p < 0.05$) with the others. When oregano EO was used, there were not significant differences ($p > 0.05$) between 12.5, 25, and 50% concentrations assayed, whereas differences were significantly different ($p < 0.05$) between 50 and 100% doses.

Table 8. Concentration Effect of Thyme, Rosemary, Marjoram, Lemongrass, Oregano, and Artemisia Essential Oils

essential oil	dose[a] (%)	diameter of inhibition zone (mm) including disk diameter of 9 mm[b] with *Listeria innocua*
Thymus vulgaris	6.25	19.50 ± 2.12 a
	12.5	26.50 ± 0.17 b
	25	33.00 ± 0.00 c
	50	40.00 ± 0.00 d
	100	40.00 ± 0.00 d
Rosmarinus officinalis	6.25	NA
	12.5	NA
	25	NA
	50	12.50 ± 0.71 a
	100	13.00 ± 0.00 a
Majorana hortensis	6.25	15.00 ± 1.41 a
	12.5	16.00 ± 1.41 a
	25	23.50 ± 0.71 b
	50	24.50 ± 0.71 b
	100	33.00 ± 0.00 c
Cymbopogon citratus	6.25	15.00 ± 1.41 a
	12.5	21.50 ± 0.71 b
	25	33.50 ± 3.54 c
	50	44.00 ± 1.41 d
	100	49.00 ± 5.66 d
Origanum syriacum	6.25	14.00 ± 1.41 a
	12.5	23.00 ± 1.41 b
	25	23.00 ± 1.41 b
	50	26.50 ± 2.12 bc
	100	29.50 ± 0.71 c
Artemisia annua	6.25	NA
	12.5	NA
	25	NA
	50	NA
	100	NA

[a] Doses of essential oil referred to initial volume (40 μL). [b] Expressed as mean ± SD. For the same essential oil, values followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple-range test. NA, nonactive.

Most of the antimicrobial activity in EOs from spices and culinary herbs appears to be associated with phenolic compounds (*38*). Chemical analysis of these EOs have shown that the principal active compounds are mainly thymol, citral, 1,8-cineole, γ-terpinene, *p*-cymene, terpinen-4-ol, and their precursors. These compounds

inhibit a variety of micro-organisms (*39*, *40*). Differences in the antimicrobial activity should be attributed to their chemical composition and relative proportions of the individual constituents in the EOs.

Burt (*39*) reported that α-terpineol, carvacrol, citral, eugenol, geraniol, perillaldehyde, and thymol have proven inhibitory effect on *L. monocytogenges*. Given the composition of the studied EOs, good inhibitory effects of thyme, oregano, lemongrass, and marjoram EOs would be expected, followed by rosemary EO and little inhibition of artemisia, as observed in **Table 8**.

Suzana et al. (*41*) reported that *T. vulgaris*, *R. officinalis*, and *O. vulgare* had anti-*Listeria* activity (*L. monocytogenes*) by using the same disk diffusion method. Marino et al. (*42*,*43*) reported that thyme and oregano EOs caused irreversible damage in *L. innocua* cells. The presence of thymol and carvacrol in thyme oil is responsible for the antimicrobial effect of the oil; those components are also present in the Egyptian thyme samples analyzed. Paparella et al. (*44*) also reported thyme and oregano EOs to inhibit *L. monocytogenes*. Pandit and Shelef (*45*) reported that rosemary EO was able to inhibit *L. monocytogenes* due to its content of α-pinene, which is also a major constituent of Egyptian rosemary EO.

Due to the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (*39*). Different modes of action have been suggested, and it can act over (i) membrane or (ii) cytoplasm.

EOs pass through the cell wall and cytoplasm membrane, disrupt the structure of their different layers of polysaccharides, fatty acids, and phospholipids, and permeabilize them. Membrane dysfunction depends on (i) interference with the energy (ATP) generation system and (ii) enzyme inhibition preventing substrate utilization for energy production (*2*, *40*, *46*).

The EOs can coagulate the cytoplasm and damage lipids and proteins. Their mechanism of action would be similar to those of other phenolics, that is, the disturbance of the proton motive force (PMF), electron flow, active transport ,and coagulation of cell contents. Instead, enzymes such as ATPases are known to be located in the cytoplasmic membrane and to be bordered by lipid molecules (*2*,*39*,*46*).

The results obtained using five different antioxidant methods (DPPH, FRAC, TBARS, FIC, and Rancimat) showed that EOs obtained from some Egyptian plants (oregano, marjoram, lemongrass, thyme, artemisia, and rosemary) can be considered good sources of natural compounds with significant antioxidant activity, which can be attributed to the high percentage of the main constituents or to synergy among the different oil constituents. Thyme, oregano, and rosemary EOs present the best antioxidant profile, whereas marjoram, lemongrass, and artemisia are highly effective in metal chelating, but all have a pro-oxidative behavior by Rancimat induction test. In addition, most of these EOs were shown to be highly effective inhibitors of *L. innocua*. Thyme and Ooregano EOs combine the best antioxidant and anti-*Listeria* effects; however, lemongrass is also of interest due to its high antimicrobial effect. The potential application of such EOs to food is of great interest because it would open an important field for the use of such natural extracts as food preservatives to replace the synthetic compounds currently used. All of these activities have been studied in vitro; therefore, their application to food is necessary to ensure that such activities are also present in food matrices.

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