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Chemical Compositions and Antioxidant/Antimicrobial Activities of Various Samples Prepared from *Schinus terebinthifolius* Leaves Cultivated in Egypt

Khaled F. El-Massry,[†] Ahmed H. El-Ghorab,[†] Hamdy A. Shaaban,[†] and Takayuki Shibamoto*,[‡]

[†]Flavor and Aroma Department, National Research Center, Cairo, Egypt, and [‡]Department of Environmental Toxicology, University of California, Davis, California 95616

Essential oil, dichloromethane extract, and ethanol extract were prepared from fresh *Schinus terebinthifolius* leaves cultivated in Egypt. The essential oil was analyzed by gas chromatography and gas chromatography/mass spectrometry. The essential oil comprised 4.97% monoterpenes, 56.96% sesquiterpenes, 34.37% oxygenated monoterpenes, and 3.32% oxygenated sesquiterpenes. The major compounds in the essential oil were *cis*-β-terpineol (GC peak area%, 17.87%), (*E*)-caryophyllene (17.56%), β-cedrene (9.76%), and citronellal (7.03%). The major phenolic compounds identified in the ethanol extract were caffeic acid (5.07 mg/100 mg extract), coumaric acid (1.64 mg), and syringic acid (1.59 mg). The antioxidant activity of ethanol extract, which was comparable with that of butylhydroquinone, was superior to essential oil and dichloromethane extract in 2,2-diphenylpicrylhydrazyl and β-carotene/bleaching assays. The dichloromethane extract exhibited the greatest antimicrobial activity against 6 strains, followed by the ethanol extract and the essential oil.

KEYWORDS: Antimicrobial activity; antioxidant activity; essential oil compositions; *Schinus terebinthifolius*

INTRODUCTION

It is well known that plants containing essential oils and various extracts may be used as alternative remedies for many infectious diseases. Recent discovery of the antimicrobial and antioxidant potential of these essential oils has extended their use as a natural preservative for prolonging the shelf life of food products (*1*, *2*). The antimicrobial activities of essential oils are also applied in pharmaceuticals, alternative medicine, and natural therapies (*3*, *4*). The high antioxidant activity found in essential oils, such as ylang−ylang, rose, and jasmine oils, has also been determined (*5*). Therefore, it is worthwhile to further investigate the medicinal components in these plants.

Antioxidants delay or inhibit the oxidation of lipid or other molecules caused by reactive oxygen species (ROS), such as free radicals, and consequently prevent oxidative damage (*6*). Oxidative damage is well known to cause various chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases (*7*). Under normal circumstances, ROS generated in the body are removed by the antioxidants, including glutathione peroxidase, catalase, and superoxide dismutase, which are naturally present in the body as well (*8*). However, endogenous antioxidant defenses are not completely efficient, and therefore, dietary antioxidants are required to diminish the cumulative effects of oxidative damage.

Schinus terebinthifolius Raddi or Brazilian pepper is a member of the Anacardiaceae family. The Brazilian peppertree, also known as Felfel Aareed, is indigenous to South and Central America and grows in semitropical and tropical regions of the United States and Africa as an ornamental garden tree. In both North and South America, three different trees (*S. molle*, *S. aroeira*, and *S. terebinthifolius)* are all interchangeably called pepper trees (*9*). The berries of pepper trees are rich in essential oil, which imparts a peppery flavor, and are used in syrups, vinegar, and beverages in Peru as well as Chilean wines. In some countries, dried and ground berries are used as a pepper substitute or as an adulterant of black pepper (*Piper nigrum*). They have also been used in the perfume industry (*10*).

Almost all parts of *S. terebinthifolius*, including leaves, bark, fruit, seeds, resin, and oleoresin (or balsam), have been used medicinally by indigenous peoples throughout the tropical regions. In South Africa, a leaf tea is used to treat colds, and a leaf decoction is inhaled for hypertension, depression, and irregular heart beat. A decoction of the bark is used in baths to relieve rheumatic and back pain (*11*). Traditionally, *S. terebinthifolius* was also used as an antibacterial, antiviral, diuretic, digestive stimulant, tonic, wound healer, anti-inflammatory, and hemostatic as well as a medicament to treat urinary and respiratory infections (*12*).

Because of the limited number of reports on the genus *Schinus*, we investigated the medicinal components of the essential oil composition and antioxidant/antimicrobial activities of the Egypt-grown *S. terebinthifolius* Raddi (*13*, *14*).

*To whom correspondence should be addressed. Tel: +1 530-752-4923. Fax: +1-530-752-3394. E-mail: tshibamoto@ucdavis.edu.

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MATERIALS AND METHODS

Chemicals and Materials. 2,2-Diphenylpicrylhydrazyl (DPPH) was purchased from TCI AMERICA (Portland, WA). Tertiary butylhydroquinone (TBHQ) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and dichloromethane (DCM) and ethanol (EtOH) were from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). All solvents were of analytical grade. The authentic compounds were bought from the above chemical companies or obtained from Takata Koryo Co., Ltd. (Osaka, Japan) as a gift.

The fresh leaves of Egyptian *S. terebinthifolius* Raddi were collected from a tree growing in five different spots in Kalubia Delta, Middle Egypt on May 2007. Leaves collected from different trees were hand mixed prior to use. Shade-dried leaves were prepared by leaving them in the shade at room temperature until dry.

Pure cultures of the bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*) and fungi (*Aspergillus niger*, *Aspergillus parasiticus*, and *Candida albicans*) used for the antimicrobial activity testings were provided by the department of Microbiology, National Research Center (NRC), Dokki, Giza, Egypt.

Preparation of Essential Oil from Fresh and Dried Leaves. The essential oil was prepared by a previously reported method (*15*). Fresh or dried *S. terebinthifolius* leaves (300 g) were cut with scissors into small pieces (approximately 0.5 mm × 0.5 mm), placed in a 3 L round-bottom flask, and mixed with 1 L of deionized water. The solution was steam-distilled for 4 h. The distillate (900 mL) was extracted with 100 mL of dichloromethane using a liquid−liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate, which was subsequently removed with filtration. The filtrate was concentrated in a rotary flash evaporator (95 mmHg and 20 °C) until the volume of the extract was reduced to approximately 1 mL. Then the solvent was further removed under a purified nitrogen stream until the volume was reduced to exactly 0.5 mL. The experiment was replicated three times.

Preparation of Dichloromethane and Ethanol Extracts from Fresh Leaves. Fresh plant leaves (200 g) were soaked in 500 mL of dichloromethane or 500 mL of 95% ethanol at room temperature for 2 days in the dark. After the solution was filtered, the solvent was removed by a rotary evaporator under reduced pressure (95 mmHg) at 20 °C for dichloromethane and 50 °C for ethanol. The condensed dichloromethane extract or ethanol extract was stored in the dark at −5 °C until further experiments. The experiment was replicated three times.

Analysis of Essential Oils. Compounds in the essential oils were identified by comparison with the Kovats gas chromatographic retention index (KI) and mass spectral fragmentation pattern of each GC component to those of authentic compounds. Identification of the GC components was also confirmed by NIST AMDIS version 2.1 software and published KI data (*16−20*).

A Hewlett-Packard model 6890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-5 bonded-phase fused-silica capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) were used. Injector and detector temperatures were 200 and 250 °C, respectively. The oven temperature was programmed from 35 to 220 °C at 3 °C/min and held for 40 min. The linear velocity of the helium carrier gas was 29 cm/s. Injections were in the split-less mode.

An HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at a MS ionization voltage of 70 eV. A 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB wax bonded-phase fused-silica capillary column (Agilent, Folsom, CA) was used for GC. The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperature was 250 °C. The oven temperature was programmed from 35 to 180 at 3 °C/min and held for 40 min.

Analysis of Phenolic Compounds. The total phenolic content of the essential oil, the dichloromethane extract, and the ethanol extract was determined by the Folin−Ciocalteu reaction method (*21*), and the results were expressed as gallic acid equivalents. Samples (0.2 mL) were mixed with 1 mL of 10-fold-diluted Folin−Ciocalteu reagent at 0.8 mL of 7.5% sodium carbonate solution. After the mixture was allowed to stand for 30 min at room temperature, the absorbance of samples was measured at 765 nm using a spectrophotometer (Shimadzu UV-1601 PC, Japan). The experiment was replicated three times.

Analysis of Ethanol Extract and Standard Compounds by Using HPLC. Analysis of ethanol extract and standard compounds were performed with an Agilent 1100 model HPLC system equipped with an Phenomenex Aqua C18 (5 μm) 250 mm × 4.6 mm i.d. column (Torrance, CA) and a diode array detection system G1315A. The mobile phase consisted of 3.5% acetic acid in water (A) and 3.5% acetic acid in methanol (B). The gradient mode was initially set at 20% B, then linearly increased to 100% B within 40 min, and held to the end (40 to 85 min). The injection volume for all samples was 100 μL. Simultaneous monitoring was performed at 280 nm at a flow rate of 1 mL/min.

DPPH Radical Scavenging Assay. The antioxidant activity of the samples was measured by the DPPH radical scavenging assay (*22*). The solution of DPPH in methanol (6×10^{-5} M) was prepared daily, immediately before UV measurements. Various concentrations of each sample (50, 100, 200, and 400 μg/ mL) were added to 1 mL of DPPH solution. The reaction mixtures were shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the samples was measured by a spectrophotometer at 517 nm. A known antioxidant, TBHQ was used to validate the assay. The experiment was replicated three times.

β-Carotene Bleaching Assay. The antioxidant activity of the samples was also examined by a β-carotene/linoleic acid system reported previously (*23*). Briefly, 1 mL of a chloroform solution of β-carotene (1 mg/mL), 40 μL of linoleic acid, and 400 μL of Tween 80 (emulgator) were placed in a round-bottom flask. After chloroform was removed under a nitrogen stream, 100 mL of distilled water was added slowly to the residue in the flask, which was subsequently agitated to give a stable emulsion. An aliquot of 4.5 mL of this emulsion was transferred to a 10 mL test tube, and then 500 μL of appropriately diluted pepper tree samples (50 to 400 μg/mL) were added in. The tubes were placed in a water bath at 50 °C, and the absorbance was measured after 20, 40, 60, 80, 100, and 120 min at 470 nm. A blank sample was prepared by adding 500 μL of distilled water to the control reaction mixtures, and the absorbance was measured immediately after preparation at 470 nm.

Antimicrobial Activity Testing. Antimicrobial activities were examined by the disk-diffusion method (*24*) using bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and fungi (*Aspergillus niger*, *Aspergillus parasiticus*, and *Candida albicans*).

Petri dishes containing the mother cultures with proper sterile media (sterile trypticase soy agar (TSA) medium was used for bacterial organisms, and potato dextrose agar (PDA) was used for fungus) were inoculated to obtain the microorganism concentration of 10^5 colony-forming units (cfu/mL). Prior to inoculation, the mother cultures of each microorganism were allowed to stand for 24 h at adequate temperature for each strain in order to reach the stationary phase of growth before the assays.

A Whatman No.1 filter paper disk (5 mm in diameter) was soaked in 100 μL each of essential oil, dichloromethane extract, or ethanol extract and allowed to stand at room temperature until dry. The disk was subsequently placed on the surface of the cold solid medium containing inoculated testing organisms in a Petri dish. After the Petri dish was incubated at 5 °C for 1 h to obtain good diffusion, it was further incubated at 37 °C for 24 h for bacteria and at 37 °C for 48 h for yeast, and 25 °C for 72 h for mold. After incubation, the degree of growth inhibition was evaluated by comparing with the controls (gentamycin sulfate and nystatin for bacteria and fungi, respectively). Each assay was performed in triplicate on three independent experimental runs.

The minimum inhibitory concentration (MIC) is defined as the minimum level of essential oil, dichloromethane extract, or ethanol extract that produces about 90% reduction in the growth (populations) of microbial colonies. The MIC was determined by the microdilution agar plate method (*25*).

Tempered TSA (90 mL) was agitated vigorously with various concentrations of essential oil, dichloromethane extract, or ethanol extract, each at (3.0, 2.8, 2.5, 2.2, 2.0, 1.5, 1.0, 0.6, 0.5, 0.25, 0.15, 0.10, 0.05, and 0.025 mL/100 mL) concentration ranges. Approximately 15 mL of each concentration mixture was transferred with 1 mL-microorganism inoculum to the agar plates. The plates were incubated for 24 h at 37 °C for bacteria, for 48 h at 30 °C for yeast, and for 72 h at 25 °C for mold. After incubation, the number of colonies in each plate was counted. Each assay was replicated three times.

Statistical Analysis. Results were expressed as the means ± standard errors ($n = 3$). One-way analysis of variance (ANOVA) was carried out to test any differences between the solvents used. Statistical comparisons between variables (e.g., yields, phenolic contents, and antioxidant/antimicrobial activities) were performed with Student's *t*-test using SPSS (version 11.0).

RESULTS AND DISCUSSION

Chemicals Identified in Essential Oils and the Effect of the Drying Process of Leaves. Yields of essential oils from leaves of Egyptian *S. terebinthifolius* were 0.50 ± 0.01 g/100 g of fresh leaves and 0.90 ± 0.02 g/100 g of shade-dried leaves (w/w). Yields of extracts from fresh leaves (water content was 60 ± 2.6%, w/w) with dichloromethane and ethanol were 1.50 ± 0.12 g/100 g and 3.40 ± 0.21 g/100 g, respectively. The values are the mean ± standard deviation ($n = 3$) ($P \leq 0.05$).

Table 1 shows compounds identified in the essential oils from Egyptian *S. terebinthifolius* leaves. As the results demonstrate, this essential oil is very rich in terpenes. The oil from fresh leaves comprises 4.97% monoterpenes, 56.96% sesquiterpenes, 34.37% oxygenated monoterpenes, and 3.32% oxygenated sesquiterpenes. The oil from dried leaves comprises 15.18% monoterpenes, 39.25 sesquiterpenes, 8.13% oxygenated monoterpenes, and 36.07% oxygenated sesquiterpenes. A total of 49 compounds were identified in the oils from fresh and dried leaves. The major compounds were *cis*-β-terpineol (17.87%), (*E*)-caryophyllene (17.56%), β-cedrene (9.76), and citronellal (7.03%) in fresh leaves and caryophyllene alcohol (13.13%), geranyl-*n*-butyrate (12.84%), β-sesquiphellandrene (8.57%), α-gurjunene (7.23%), and β-cedrene (7.23%) in dried leaves. Most of these chemicals play important roles in the aromas of essential oils. For example, caryophyllene possess a woody-spicy odor with a somewhat bitter taste and has been used particularly for chewing gum as well as in spice blends and flavor compositions (*26*).

The results indicate that the relative composition of some components changed significantly by drying. The components increased by drying were santolina triene (trace to 5.83%), sabinene (1.2 to 7.63%), and geranyl-*n*-butyrate (0.51 to 12.21%). The components decreased were *cis*-β-terpineol (17.87 to 3.65%) and (*E*)-caryophyllene (17.56 to 1.59%). The total oxygenated sesquiterpenes exhibited the most significant increase by drying (3.32% to 36.07%). However, total oxygenated monoterpenes decreased from 34.37% to 8.13%. Volatile aroma compounds are the most sensitive components in the process of food drying. The effect of drying on the composition of volatile flavor constituents of various aromatic plants and vegetables has been the subject of numerous studies (*27*). The loss of volatiles in herbs and spices during drying depends mainly on drying conditions and the biological characteristics of plants. Some compounds evaporate during drying, whereas others are partially retained, and others arise as oxidation products during drying (*28*). The antioxidant activity of essential oils was also affected by drying (*29*).

Phenolic Compounds Determined in Fresh Leaves. The total concentration of phenolic compounds was 7.00 ± 0.02% in the essential oil, 22.00 ± 0.51% in the dichloromethane extract, and 35.00 ± 0.75% in the ethanol extract. Values are the mean ± SD ($n = 3$, w/w). Phenolic compounds identified in the ethanol extract from fresh leaves were caffeic acid (5.07 ± 0.01 mg), syringic acid (1.59 ± 0.00 mg), coumaric acid (1.64 ± 0.05 mg), ellagic acid (0.54 ± 0.00 mg), gallic acid (0.27 ± 0.00 mg/100 g), and catechin (trace). Values are the mean ± SD ($n = 3$)/(100 g of fresh leaves).

Typical phenolic compounds known to possess antioxidant activity are mainly phenolic acids and flavonoids (*30*). For example, gallic acid, caffeic acid, ellagic acid, syringic acid, coumaric acid, and catechin are widely distributed in the plant kingdom (*31*).

Table 1. Chemical Composition of Essential Oils from Egyptian *Schinus terebinthifolius* Leaves

		GC peak area %[a]	
compound	I[b]	fresh	dry
	Nonterpenes		
pentanal	697	–[c]	0.30
ethyl propanoate	714	–	0.67
1-octene	792	–	1.88
	Monoterpenes		
5-methylenenorbonene	803	–	0.54
santolina triene	908	–	5.83
α-pinene	939	1.00	–
α-fenchene	951	0. 83	–
verbenene	967	0.52	–
sabinene	976	1.20	7.63
m-cymenene	1082	0.4	–
terpinolene	1088	1.02	–
	Monoterpenoids		
trans-thujone	1112	1.53	0.55
camphor	1143	0.65	1.81
cis-β-terpineol	1144	17.87	3.56
neo-3-thujanol	1148	2.67	–
citronellal	1149	7.03	1.24
(*Z*)-ocimenone	1228	0.55	–
(*E*)-ocimenone	1231	0.95	–
methyl citronellate	1250	0.92	–
neo-3-thujyl acetate	1267	0.43	–
dehydroelsholtza ketone	1287	0.4	–
	Sesquiterpenes		
α-cubebene	1354	0.58	–
cyclosativene	1358	0.79	–
α-copaene	1376	1.87	–
patchoulene	1380	2.48	1.01
β-bourbonene	1384	2.74	0.27
isolongifolene	1387	3.45	0.28
β-elemene	1391	0.73	–
cyperene	1394	1.25	0.61
α-gurjunene	1398	1.76	7.23
α-cedrene	1409	1.31	4.45
β-cedrene	1409	9.76	7.23
(*E*)-caryophyllene	1418	17.56	1.59
dehydroaromadendrene	1459	0.74	1.35
γ-cadinene	1521	3.39	1.60
7-epi-α-selinene	1513	1.48	1.02
γ-cadinene	1517	3.69	1.50
β-sesquiphellandrene	1524	3.17	8.57
(*E*)-γ-bisabolene	1531	1.18	1.34
selina-3,7-diene	1542	0.4	1.19
sesquisabinene hydrate	1547	–	0.61
	Sesquiterpenoids		
6,11-oxidoacor-4-ene	1525	2.04	1.26
α-agarofuran	1546	–	0.73
geranyl-*n*-butyrate	1549	0.51	12.21
Ledol	1552	–	3.02
caryophyllene alcohol	1555	0.77	13.13
spathulenol	1563	–	3.49
globulol	1569	–	2.03
dihydro-ar-turmerone	1586	–	0.27

[a] Solvent peak is excluded. [b] Kovat's index on DB-5. [c] Not detected, or less than 0.01%.

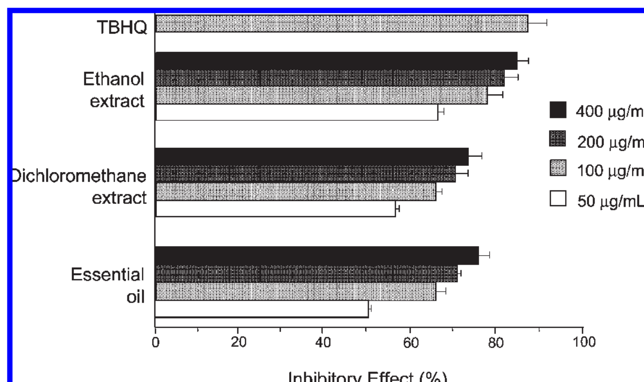

Figure 1. Antioxidant activity of samples from fresh leaves examined using the DPPH assay.

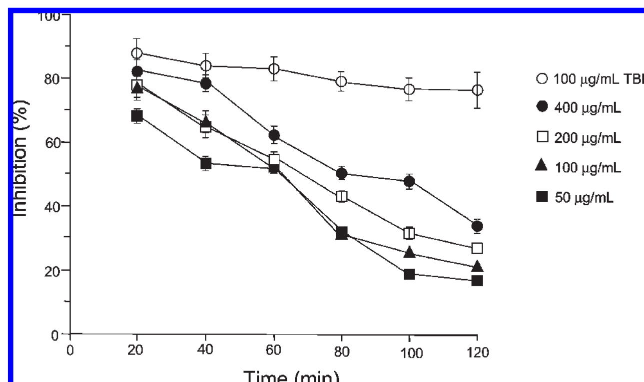

Figure 3. Typical relationship between inhibitory activity and time obtained with the essential oil using the β-carotene bleaching assay.

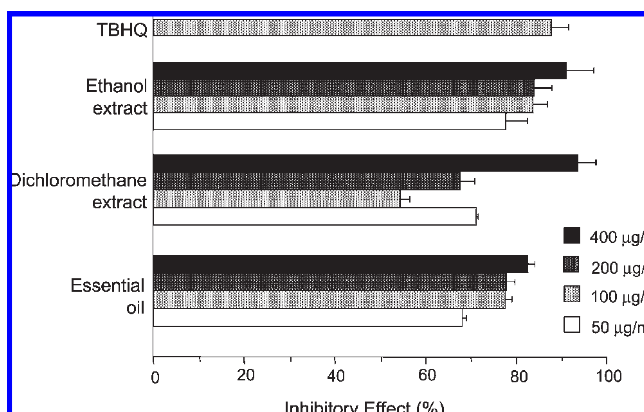

Figure 2. Antioxidant activity of samples from fresh leaves examined using the β-carotene bleaching assay measured at 20 min.

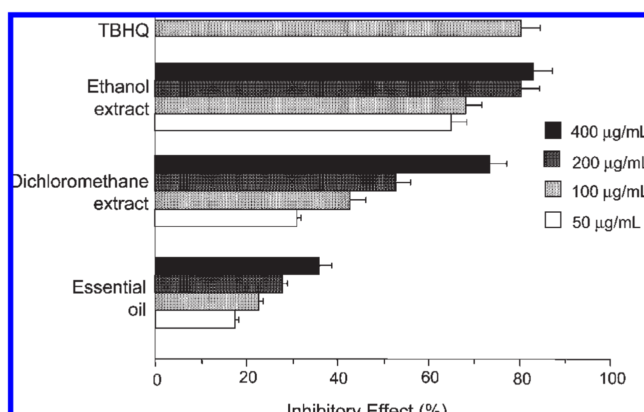

Figure 4. Antioxidant activity of samples from fresh leaves examined using the β-carotene bleaching assay measured at 120 min.

Antioxidant Activities of Samples. It is well known that plants contain various antioxidants such as ascorbic acid, tocopherols, polyphenolic compounds, and terpenoids (*32*). In particular, phenolic compounds have received much attention as one of the principle antioxidants found in plants (*33*). In addition, recent reports indicate that some essential oils and organic solvent extracts from plants possess antioxidant activity (*5*, *34*).

Results of DPPH Assay. The antioxidant activity of essential oil, dichloromethane extract, and ethanol extract from fresh leaves was examined using free radical scavenging capacities of the samples by DPPH assay. The results are shown in **Figure 1**. A known antioxidant, TBHQ was used to validate this assay. The inhibition of TBHQ was 86.4 ± 4.0% at a level of 100 μg/mL. All samples tested exhibited antioxidant activity with dose response. Among them, the ethanol extract exhibited the most potent antioxidant activity. It showed 84.1 ± 3.2% and 65.7 ± 2.0% inhibitory effects at levels of 400 μg/mL and 50 μg/mL, respectively. The essential oil and dichloromethane extract showed a similar inhibitory effect, a slightly lower level than that of the ethanol extract. Both samples exhibited quite appreciable antioxidant activities. For example, the essential oil and the dichloromethane extract showed 75.2 ± 2.0% and 72.7 ± 3.0% inhibitory effects at a level of 400 μg/mL, respectively.

Results of the β-Carotene Bleaching Assay. **Figure 2** shows the results after 20 min. All samples exhibited satisfactory antioxidant activity with clear dose responses except in the case of the dichloromethane extract, in which 50 μg/mL (71.2 ± 1.3%) showed a higher effect than 100 (54.2 ± 2.5%) and 200 μg/mL (67.3 ± 3.4%). The standard compound, TBHQ showed strong inhibitory activity (87.2 ± 4.2%) at a level of 100 μg/mL, indicating that this method is valid. The ethanol extract and dichloromethane extract showed stronger activity than that of TBHQ at the level of 400 μg/mL (90.1 ± 5.3% and 93.6 ± 4.7%, respectively).

The bleaching activity decreased gradually over time. **Figure 3** shows the typical relationship between inhibitory activity and time obtained with the essential oil. **Figure 4** shows the results after 120 min. All samples exhibited antioxidant activity with clear dose response. The ethanol extract and dichloromethane extract exhibited potent antioxidant activity at a level of 400 μg/mL (78.2 ± 4.9% and 69.2 ± 3.5%, respectively). The essential oil showed moderate activity.

The results of the present study indicate that various antioxidants are present in the samples obtained from Egyptian *S. terebinthifolius* leaves. Among the samples tested, the ethanol extract exhibited the most potent antioxidant activity. This may be due to the presence of phenolic compounds in the ethanol extract (*35*, *36*) because the dichloromethane extract and essential oil contained a lower concentration of phenolic contents in comparison with that of the ethanol extract. However, both samples exhibited comparable activities to those of the ethanol extract at a high level (400 μg/mL). This phenomenon may be due to the presence of terpenes with antioxidant activity, such as terpineol, caryophyllene, cedrene, sabinene, terpinolene, and camphor, with strong antioxidant activity (*37*).

Antimicrobial Activity of Samples from Fresh Leaves. The results of antimicrobial activity tests are shown in **Tables 2** and **3**. All the samples from *S. terebinthifolius* exhibited inhibitory activity against the microorganisms used. The low activity observed against *E. coli* may be due to lipopolysaccharides in the outer membrane of the G- negative bacteria, which make

Table 2. Antimicrobial Activity of Samples from Egyptian *Schinus terebinthifolius* Leaves against Various Strains

sample	Inhibition zone (mm)					
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus niger*	*Aspergillus parasiticus*	*Candida albicans*
essential oil	17	19	5	15	11	16
ethanol extract	19	21	8	16	13	17
dichloromethane extract	20	23	10	18	15	19
gentamycin sulfate	35	40	29			
nystatin (50 IU)				38	29	30

Table 3. Minimum Inhibitory Concentration (MIC) of Samples from Egyptian *Schinus terebinthifolius* Leaves against Various Strains

sample	MIC (mg/mL)					
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus niger*	*Aspergillus parasiticus*	*Candida albicans*
essential oil	0.80	0.80	1.10	0.85	0.90	0.85
ethanol extract	0.75	0.75	0.95	0.80	0.85	0.80
dichloromethane extract	0.60	0.55	0.85	0.75	0.80	0.7

them inherently resistant to external agents, such as hydrophilic dyes, antibiotics, and detergents (*38*).

The results obtained from the disk diffusion method, followed by measurements of minimal inhibition concentration (MIC), indicate that the dichloromethane extract showed particularly strong inhibition in the growth of strains *Pseudomonas aeruginosa* (23 mm with MIC of 0.55 mg/mL), *Staphylococcus aureus* (20 mm with 0.6 mg/mL), *Aspergillus niger* (18 mm with 0.85 mg/mL), *Aspergillus parasiticus* (15 mm with 0.8 mg/mL), and *Candida albicans* (19 mm with 0.75 mg/mL). The essential oil and ethanol extract showed only moderate activity.

Generally, the antimicrobial activities of essential oils have been accounted for by positing that monoterpenes and sesquiterpenes with aromatic rings and phenolic hydroxyl groups form hydrogen bonds with the active site of target enzymes (*39*). Therefore, the antimicrobial activity of the essential oil from *S. terebinthifolius* fresh leaves could be ascribed to the presence of biologically active compounds, including *cis*-β-terpineol (17.87%), citronellal (7.03%), neo-3-thujanol (2.67%), neo-3-thujyl acetate (2.67%), methyl citronellate (0.92%), and caryophyllene alcohol (0.77%).

In the previous study, 49 extracts, including the ethanol extract, from the stem bark of 25 plants of Brazilian traditional medicine exhibited antibacterial activities against *E. coli* and *S. aureus* strains. (*40*). However, there are no reports on the antimicrobial activity of samples obtained from *S. terebinthifolius* fresh leaves grown in Egypt.

The results of the present study suggest that Egyptian *S. terebinthifolius* could be a source of natural antioxidant/antimicrobial supplementation, in addition to its use as a flavor ingredient for various food products.

LITERATURE CITED

(1) Dorman, H. J.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316.
(2) Bruni, R.; Medici, A.; Andreotti, E.; Fantin, C.; Muzzoli, M.; Dehesa, M.; Romagnoli, C.; Sacchetti, G. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (*Lauraceae*) flower calices. *Food Chem.* **2004**, *85*, 415–421.
(3) Reynolds, J. E. *Martindale the Extra Pharmacopeia*, 31st ed.; Royal Pharmaceutical Society of Great Britain: London, 1996.
(4) Lis- Balchin, M.; Deans, S. G. Bioactivity of selected plant essential oils against Listeria monocytogenes. *J. Appl. Bacteriol.* **1997**, *82*, 759–762.
(5) Wei, A.; Shibamoto, T. Antioxidant activities and volatile constituents of various essential oils. *J. Agric. Food Chem.* **2007**, *55*, 1737–1742.
(6) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117.
(7) Halliwell, B.; Grootveld, M. The measurement of free radical reactions in humans. *FEBS Lett.* **1987**, *213*, 9–14.
(8) Aruoma, O. I. Nutrition and health aspects of free radicals and antioxidants. *Food Chem. Toxicol.* **1994**, *32*, 671–683.
(9) Campelo, J.; Marsaioli, A. J. Triterpenes of Schinus terebinthifolius. *Phytochemistry* **1974**, *13*, 659–660.
(10) Moneam, N. M. A.; Ghoneim, T. Gas chromatographic analysis of total 1 fatty acids extracted from *S. terebinthifolius* berries. *J. Chromatogr.* **1986**, *361*, 391–395.
(11) Panetta, F. D.; McKee, J. Recruitment of the invasive ornamental, *S. terebinthifolius*, is dependent upon frugivores. *Aust. J. Ecol.* **1997**, *22*, 432–438.
(12) Melo-Junior, E. J.; Raposo, M. J.; Lisboa, N. J. A.; Diniz, M. F. A.; Marcelino, C. A. C.; Santana, A. E. G. Medicinal plants in the healing of dry socket in rats: microbiological and microscopic analysis. *Phytomedicine* **2002**, *9*, 1109–1116.
(13) Martinez, M. J.; Betancourt, J.; Alonso-Gonzales, N.; Jaurequi, A. Screening of some Cuban medicinal plants for antimicrobial activity. *J. Ethnopharamcol.* **1996**, *52*, 171–174.
(14) Erazo, S.; Delporte, C.; Negrete, R.; Garcia, R.; Zaldivar, M.; Iturra, G.; Caballe, E.; Lopez, L.; Backhouse, N. Constituents and biological activities of *S. polygamus*. *J. Ethnopharmacol.* **2006**, *107*, 395–400.
(15) Lee, K. G.; Shibamoto, T. Inhibition of malonaldehyde formation from blood plasma oxidation by aroma extracts and aroma components isolated from clove and eucalyptus. *Food Chem. Toxicol.* **2001**, *39*, 1199–1204.
(16) Pagula, F. P.; Baser, K. H. C.; Kurkcuoglu, M. Essential oil composition of *Eucalyptus camaldulensis Dehn.* from Mozambique. *J. Essent. Oil Res.* **2003**, *12*, 333–335.
(17) Iqbal, Z.; Hussain, I.; Hussain, A.; Ashraf, M. Y. Genetic variability to essential oil contents and composition in five species of *eucalyptus*. *Pak. J. Bot.* **2003**, *35*, 843–852.
(18) Bhatti, H. N.; Iqbal, Z.; Chatha, S. A. S.; Bukhari, I. H. Variations in oil potential and chemical composition of *Eucalyptus crebra* among different districts of Punjab−Pakistan. *Intern. J. Agric. Biol* **2007**, *9*, 136–138.
(19) Fadel, H. H. M.; Marx, H.; Al-Sawy, A.; El-Ghorab, A. Effect of extraction techniques on the chemical compositions and antioxidant activity of *Eucalyptus camaldulensis* Var. *brevirostris* leaf oils. *Z. Lebensm.-Unters-Forsch.* **1999**, *208*, 212–216.
(20) El-Ghorab, A.; Fadel, H. H. M.; El-Massry, K. F. The Egyptian *Eucalyptus Camaldulensis* Var. *Brevirostris*: Chemical compositions of fruits volatile oil and antioxidant activity. *Flav. Fragr. J.* **2002**, *17*, 306–312.
(21) Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K. Antioxidant activity of grape seeds (*Vitis vinefera*) extracts on peroxidation models *in vitro*. *Food Chem.* **2001**, *73*, 285–290.

(22) Miliauskas, G.; Venskutonis, P. R.; Beek, T. A. Screening of radical scavenging activity of some medical and aromatic plant extracts. *Food Chem.* **2004**, *85*, 231–237.
(23) Matthaus, B. Antioxidant activity of extracts obtained from residues of different oilseeds. *J. Agric. Food Chem.* **2002**, *50*, 3444–3452.
(24) Black, J. G. Antimicrobial Agents from Higher Plants. I. Introduction, Rational and Methodology, Lloydia. In *Microbiology Principles and Explorations*, 5th ed.; John Wiley and Sons, Inc.: New York, 2002; pp 336−366.
(25) Skandamis, P.; Koutsouumanis, K.; Fasseas, K.; Nychas, G. J. Inhibition of oregano essential oil and EDTA on *E. coli* 0157:H. *Ital. J. Food Sci.* **2001**, *13*, 55–65.
(26) Arctander, S. *Perfume and Flavor Chemicals*; Steffen Arctander: Montclair, NJ, 1969; p 583.
(27) Omidbaigi, R.; Sefidkon, F.; Kazemi, F. Influence of drying methods on the essential oil content and composition of Roman camomile. *Flav. Fragr. J.* **2004**, *19*, 196–198.
(28) Luning, P. A.; Ebbenhorstseller, T.; Derijk, T.; Rozen, J. P. Effect of hot air drying on flavour compounds of bell peppers (*Capsicum annuum*). *J. Sci. Food Agric* **1995**, *68*, 355–365.
(29) Chin- Lin, H.; Wenlung, C.; Yih-Ming, W; Chin-Yin, T. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. *Food Chem.* **2003**, *83*, 85–92.
(30) Kahkoenen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954–3962.
(31) Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **1988**, *27*, 969–978.
(32) Grassmann, J. Terpenoids as plant antioxidants. *Vitam. Horm.* **2005**, *72*, 505–535.
(33) Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157–2184.
(34) El-Ghorab, A.; El-Massry, K. F.; Shibamoto, T. Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (*Zea mays L.*). *J. Agric. Food Chem.* **2007**, *55*, 9124–9127.
(35) Liu, R.; Li, A.; Sun, A.; Cui, J.; Kong, L. Preparative isolation and purification of three flavonoids from the Chinese medicinal plant *Epimedium koreamum* Nakai by high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1064*, 53–57.
(36) Montoro, P.; Braca, A.; Pizza, C.; De Tommasi, N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem.* **2005**, *92*, 349–355.
(37) Ruberto, G.; Bratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* **2000**, *69*, 167–174.
(38) Negi, P. S.; Jayaprakasha, G. K. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *J. Food Sci.* **2003**, *68*, 1473–1477.
(39) Sivropoulou, A.; Kokkini, S.; Lanaras, T.; Arsenikas, M. Antimicrobial activity of mint essential oils. *J. Agric. Food Chem.* **1995**, *43*, 2381–2388.
(40) De-Lima, F. M.; De-Souza, L.; dos-Santos, J.; De-Andrade, A. F.; Ana, M. C.; Moreau, N. Anti- bacterial activity of some Brazilian medicinal plants. *J. Ethnopharmacol.* **2006**, *105*, 137–147.

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