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Aroma Volatiles, Antibacterial, Antifungal and Antioxidant Properties of Essential Oils Obtained from Some Spices Widely Consumed in Egypt

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Abstract: The aim of this study was determine the volatile compounds, antimicrobial and antioxidant activity of the essential oils (EOs) of cinnamon (*Cinnamomum verum*), fenugreek seeds (*Trigonella foenum greacum*), ginger (*Zingiber officinale*) and marjoram (*Origanum majorana*) widely consumed in Egypt. EOs showed a great variety of compounds in their chemical compositions, *Trans*-cinnamyl aldehyde, terpinene-4-ol, geranial and linolenic acid were the predominant aroma volatiles in cinnamon, marjoram, ginger and fenugreek with 57.37%, 36.6%, 32.75% and 24.28%, respectively. Total phenolic content (TPC) and total flavonoid content (TFC) as well as antioxidant activity were also determined of four Egyptian aromatic plants. Ethanolic extract of cinnamon had the highest TPC and TFC while fenugreek methanolic extract had the lowest value. Cinnamon had the highest antioxidant profile, which was verified in DPPH and ABTS assays. Cinnamon and fenugreek EOs showed the highest antifungal activity against *Fusarium moniliforme, Aspergillus flavus* and *Aspergillus ochraceus.* Fenugreek and cinnamon oils showed maximum antibacterial activity against *six bacterial strains*. In conclusion, all tested essential oils have very strong potential applicability as antibacterial, antifungal and antioxidant agents, specially cinnamon oil, which could be applied as additives in food and pharmaceutical industries.

Key words: Volatile Oils · Antioxidant Activity · Polyphenols · Antimicrobial Activity

INTRODUCTION

Spoilage and poisoning of food by fungi are a major problem in developing countries especially in Egypt. *Penicillium, Aspergillus* and *Fusarium* are the most important fungi causing spoilage of foodstuffs. Fungi are also responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses. The use of essential oils (EOs) and plant extracts as well as other alternative forms of medicinal treatments find great popularity all over the world. Many plants especially aromatic herbs are recommended to be used as food preservatives and functional food additives [1]. Saggiorato *et al*.[2] reported that presence of spoilage microorganism in food can accelerate the oxidation processes and produce changes in the organoleptic properties, specially fungi that can give some characteristic color to the food. They are also directly responsible for the development of illnesses in the human organs or can be indirectly responsible due to the production of toxins; e.g. mycotoxins, produced by fungi. In food industries sector, some synthetic additives were used as antimicrobial and antioxidant agents to prevent food deterioration and microbial growth. These synthetic additives can cause DNA damage and cancer. Essential oils (Eos) have shown antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties [3]. In addition, EOs, are gaining interest for their potential as preservatives and decontamination agents, since such substances have been recognized as safe and are widely accepted by consumers. However, these properties are not always detectable with the same efficiency in all

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plants, even in the same species. This occurs because the efficiency of the EOs depends on its chemical composition and the composition depends on the genotype of the plant as well as on the environmental and agronomic conditions. There is an increasing interest to use of antimicrobial and other drugs derived from plants instead of the synthesized products. Initial screening of potential antibacterial and antifungal compounds from plants could be performed usage pure substances or crude extracts [4]. The chemical composition and biological properties (antimicrobial and antioxidant activities) of EOs of cinnamon, marjoram, ginger and fenugreek had been studied. However, there are only limited data on the composition and antimicrobial and antioxidant activities of EOs obtained from these aromatic plants cultivated in Egypt. Thus, the aim of this study was to determine (i)chemical composition (ii) antimicrobial activity(iii) antioxidant activity of cinnamon, fenugreek seeds, ginger and marjoram widely used in Egypt.

MATERIALS AND METHODS

Plants and Essential Oil Extraction: Cinnamon (Stem bark), fenugreek (dried seeds), ginger (fresh rhizomes), marjoram (fresh whole plant) were used in this work, common traditional use and zonal production in Egypt, were purchased from the specialized local market. The essential oils were extracted by the method using Clevenger's apparatus [5]

Gas Chromatography mass Spectrometry (GC/MS): Essential oils of cinnamon, ginger, marjoram and fenugreek were analyzed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220°C and 250°C, respectively. One microliter essential oil solution was injected and analyzed with the column held initially at 60°C for 2 min and then increased by 3°C/min up to 300°C. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by linear retention indices (RI) and mass spectra [6].

Preparation of Ethanol and Methanol Extracts: Each aromatic plant was extracted in ethanol or methanol (95%) [7].

Determination of total phenolic compounds (TPC) and total flavonoid content (TFC) were determined as described by Dewanto *et al*.[8]. Results of TPC were expressed as µg/mL of gallic acid equivalents (GAE) / g. The calculation of TFC was carried out using Catechin calibration curve.

In Vitro* Antioxidant Activity** ***:(i) DPPH scavenging assay: Various concentrations of essential oil, methanol and ethanol extracts were used as described by Hanato *et al*. [9]. (ii) *ABTS radical cation decoulorization assay :* $ABTS^{\cdot}$ radical scavenging activity of EOs, ethanol and methanol extracts were determined according to Re *et al*. [10]. The absorbance of the resulting oxidized solution was compared as µM trolox equivalents/g dry weight.

Microorganisms and Cultures: The cultures used in this study were obtained from the Mycotoxins Lab, Department of Food Toxicology and contaminants, National research Centre, Egypt. The fungal strains of *F.moniliform* (IBT 9490), *A. flavus* and *A. ochraceus* (local strain) which were identified as aflatoxins and ochratoxin-The producing strains were obtained and subcultured in nutrient Yeast Extract Sucrose media (YES). Sensitivity agar test was used in antifungal sensitivity testing. Simultaneously, the bacterial strains of *Pseudomonas aeruginosa* (NCIB 950), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCIB 3610), *Klebsiella pneumoniae* (NCIB 418), *Escherichia coli* (NCIB 86) and *Proteus vulgaris* were obtained and subcultured in tryptone Soya Agar media (TSA). The inoculated YES and TSA media were incubated 28 ± 1°C for 10 days and 37 ± 1°C for 24 hours, respectively, before sensitivity testing and evaluating the efficacy of the studied plant essential oils.

Preparation of the Conidial Suspension: Conidia were harvested from 7-day-old cultures by pouring a sterile 0.01% aqueous solution of Tween 80 on to the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia was adjusted to approximately 106 conidia /ml using a Burker-Turk counting chamber (Heamocytometer).

Antifungal Properties of Essential Oils: Essential oils were assayed for their antifungal activities on the radial growth of mycelia, using agar dilution method [11].

Antibacterial Assay by Disk Diffusion Method: Screening of essential oils for antibacterial activity was done by the disk diffusion method, which is normally used as a preliminary check and to select between efficient essential oils [3].

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) is defined as the minimum level of essential oil concentration that produces a 90% reduction in the growth (populations) of microbial colonies [12]. Inhibition of bacterial growth in the plates containing test oil was judged by comparison with growth in blank control plates.

Statistical Analysis: Results were expressed as means (±standard deviation n=3). Statistical analysis was performed using SPSS statistical program for windows (Version 16). All data were statistically analyzed using analysis of variance and the results were considered significant at $P < 0.05$.

RESULTS AND DISCUSION

Chemical Composition of the Essential Oils: Table 1 shows the chemical constituents, their relative percentage of the total chromatogram area and linear retention indices of cinnamon, marjoram, ginger and fenugreek EO. In the cinnamon EO there were identified 18 compounds, which represent 97.46 % of the total EO. The major compounds were trans-cinnamyl aldehyde (57.37%), L-bornyl acetate (13.06%), caryophyllene oxide (5.82%) and 1-hexadecanol (5.40%). These results are in agreement with Wang *et al*. [13] who reported that the major compound in the cinnamon EO was trans-cinnamyl aldehyde. When the EO of marjoram was analyzed, 27 compounds were identified, representing 97% of the total EO. The main components were terpinene-4-ol (36.60%), Linalool (13.58%), β-Phyllandrene (6.57%) and cis-thujanol (6.13%). Abd El-Moneim *et al*. [14] reported that the main component in marjoram volatile oil was Terpinene-4-ol with 23.86 %. The volatile oil of ginger rhizomes of this

Table 1: Aroma volatiles of the essential oils.

		Relative area %				
Identified compounds [a]	LRI	Cinnamon	Marjoram	Ginger	Fenugreek	Method of Identification[b]
Heptenal	----	0.33	ND	ND	ND	
2-Methyl-2-butenal	<800	ND	ND	ND	0.32	RI, MS & St
Pyridine	<800	ND	ND	ND	0.21	RI, MS & St
Furfuryl alcohol	883	ND	ND	ND	0.24	RI &MS
2-Heptanone	888	ND	ND	ND	0.12	RI &MS
2-Ethylpyrazine	912	ND	ND	ND	0.65	RI &MS
α-Thujene	923	ND	0.15	ND	ND	RI &MS
Tricyclene	927	ND	0.61	ND	ND	RI &MS
α - Pinene	930	ND	0.99	ND	ND	RI &MS&St
β-Pinene	942	ND	1.5	2.16	ND	RI &MS&St
Carene (?)	945	ND	0.11	ND	ND	RI &MS
Camphene	948	ND	0.27	1.58	ND	RI &MS
5-Methylfurfural	966	ND	ND	ND	0.21	RI &MS
Myrcene	994	ND	ND	ND	0.12	RI &MS
Trimethylpyrazine	1014	ND	ND	ND	0.08	RI &MS
O-Cymenene	1016	ND	0.5	ND	ND	RI &MS
α-Terpinene	1018	ND	ND	0.96	ND	RI &MS
Caproic acid	1019	ND	ND	ND	0.85	RI &MS
Limonene	1020	ND	4.77	ND	ND	RI &MS&St
β-Phyllandrene	1023	ND	6.57	ND	ND	RI &MS
Acetic acid	1033	ND	ND	ND	0.2	RI &MS
(*E*)-2-Heptenal	1041	ND	ND	0.28	ND	RI &MS
1,8-Cineol	1051	ND	4.36	ND	ND	RI, MS & St
α-Terpinene	1062	ND	5.21	ND	ND	RI, MS & St
Cis-Sabinene hydrate	1070	ND	ND	7.85	ND	RI &MS
P-Cymene	1073	ND	1.47	ND	ND	RI &MS
Cis-Thujanol	1078	ND	6.13	ND	ND	RI &MS
3-Ethyl-2,5-dimethylpyrazine	1083	ND	ND	ND	0.38	RI &MS
Terpinolene	1091	ND	5.0	ND	ND	RI &MS

Table 1: Continue

Identified compounds [a]	LRI	Relative area %				Method of Identification[b]
		Cinnamon	Marjoram	Ginger	Fenugreek	
Trans- Sabinene hydrate	1093	ND	2.9	ND	ND	RI &MS
Carvone	1097	ND	0.64	ND	0.78	RI &MS
Linalool	1103	ND	13.58	ND	ND	RI &MS
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	1112	ND	ND	ND	0.75	RI &MS
Sotolon	1122	ND	ND	ND	3.28	RI &MS
Terpinene-4-ol	1190	ND	36.6	ND	ND	RI &MS
Iso-bronyl acetate	1204	ND	0.09	ND	ND	RI &MS
α-Terpineol	1206	ND	5.87	ND	ND	RI &MS
Coumaran	1241	ND	ND	ND	1.2	RI &MS
Geranial	1270	ND	ND	32.75	ND	RI &MS
trans-Cinnamyl aldehyde	1274	57.37	ND	ND	ND	RI &MS
Cinnamayl alcohol	1280	1.39	ND	ND	ND	RI &MS
L-Bornyl acetate	1289	13.06	ND	ND	ND	RI &MS
Thymol	1311	ND	1.11	ND	ND	RI &MS
Caryophyllene	1329	ND	0.07	ND	ND	RI &MS&St
Terpinyl acetate	1337	ND	0.34	ND	ND	RI &MS
Eugenol	1360	2.26	ND	ND	ND	RI &MS
Geranyl acetate	1384	ND	ND	1.18	ND	RI &MS&St
α-Muurolene	1481	0.43	ND	ND	ND	RI &MS
α-Copaene	1383	1.22	ND	ND	ND	RI &MS
β-Caryophyllene	1426	2.42	ND	ND	ND	RI &MS
Hummlene	1441	ND	1.25	ND	ND	RI &MS
Trans-Cinnamyl acetate	1446	1.3	ND	ND	ND	RI &MS
α-Humuleneα	1449	2.82	0.07	ND	ND	RI &MS
curcumene	1481	ND	ND	0.78	ND	RI &MS
β-Bisabolene	1483	ND	ND	ND	ND	RI &MS
Germacrene(D)	1486	ND	ND	2.2	ND	RI &MS
α-Zingiberene	1495	ND	ND	14.35	ND	RI &MS
ä-Cadinene	1509	0.29	ND	ND	ND	RI &MS
β-sesquiphellandrene	1523	ND	ND	9.43	ND	RI &MS
Trans-Nerolidol	1556	ND	ND	0.18	ND	RI &MS
Caryophyllene oxide	1560	5.82	ND	ND	ND	RI &MS
Spathulenol	1566	ND	0.39	ND	ND	RI &MS
Caryophyllene oxide	1588	ND	ND	3.56	ND	RI &MS
Trans- Muurolene	1620	0.24	ND	ND	3.28	RI &MS
α-Cadinol	1635	0.28	ND	ND	ND	RI &MS
γ-Eudesmol	1653	ND	ND	15.95	ND	RI &MS
β-Eudesmol	1654	ND	ND	1.29	ND	RI &MS
β-Bisabolol	1657	0.64	ND	ND	ND	RI &MS
Epi-α-Bisabolol	1692	0.43	ND	0.45	ND	RI &MS
Benzyl benzoate	1710	1.8	ND	ND	ND	RI &MS
Trans-Farnesol	1725	ND	ND	0.77	ND	RI &MS
Tetradecanoic acid	1779	ND	ND	ND	6.2	RI &MS
1-Hexadecanol	1865	5.4	ND	ND	ND	RI &MS
Phytol	1956	ND	1.02	1.15	ND	RI &MS&St
Hexadecanoic acid	1950	ND	ND	ND	9.2	RI &MS
Linoleic acid	2095	ND	ND	ND	22.65	RI &MS
Octadecanoic acid	2126	ND	ND	ND	6.34	RI &MS
Linolenic acid	2163	ND	ND	ND	24.28	RI &MS
Oleic acid	2175	ND	ND	ND	15.23	RI &MS&St
Total volatiles		97.46	97.07	96.87	96.57	

a: Compound listed in the order of elution from a DB_5 column;

RI: Retention indices relative to C_7-C_{20} *n*-alkanes on the DB-5MS column;

b: identification based on retention index; MS, identification based on comparison of mass spectra

Table 2: Antioxidant activity, Total phenolic content (TPC) and Total flavonoid content (TFC) of cinnamon, fenugreek, marjoram and ginger.

Plant	DPPH assay (Inhibition %)						
Extract	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	ABTS assay (µm trolox / g)	TPC (mg GAE/g)	TFC (mg CE/g)
CEO	38.4±2.1	52.6±4.5	72.5±6.2	89.6±5.23	1023±51.3	--	--
CEE	32.4±3.4	47.2±3.4	67.1±4.1	83.1±4.07	983.2±45.3	284.9±6.8	78.3±2.8
CME	27.5±2.5	45.3±2.9	60.7±4.3	75.1±5.02	868.4±44.1	239.4 ±8.5	53.4±3.4
FEO	18.94±1.5	30.56±2.7	44.3±3.7	62.34±3.9	33.2±2.2	-------	----------
FEE	20.3±1.8	39.4±4.1	50.2±4.2	65.4±4.3	32.8±1.9	89.42±4.8	39.4±2.4
FME	17.5±1.6	35.7±3.2	47.2±3.3	60.9±4.1	24.3±2.1	72.9±2.9	25.83±2.7
MEO	30.2±2.2	48.9±3.3	64.4±4.9	77.6±5.2	945.4±50.1	-------	----------
MEE	32.5±2.9	52.1±3.9	66.2±5.1	79.2±7.0	854.3±42.7	256±5.3	68.12±3.1
MME	28.9±3.1	48.5±4.0	63.2±4.7	74.6±4.06	786.4±39.4	220.3±4.2	58.32±2.7
GEO	35.4±2.5	51.4±3.8	62.8±3.9	79.4±5.7	989.7±44.2	-----	-------
GEE	33.2±1.9	48.1±2.8	69.4±4.2	80.1±3.9	895.2±42.5	231±3.8	56.87±3.6
GME	27.6±1.8	45.6±3.0	63.5±3.8	75.3±4.8	778.4±33.5	189±3.65	45.63±2.8

CEO: Cinnamon essential oil, CEE: Cinnamon ethanol extract; CME: Cinnamon methanol extract; FEO: Fenugreek essential oil; FEE: Fenugreek ethanol extract; FME: Fenugreek methanol extract; MEO: Marjoram essential oil; MEE: Marjoram ethanol extract; MME: Marjoram methanol extract; GEO: Ginger essential oil; GEE: Ginger ethanol extract and GME: Ginger methanol extract. mg CE/g: mg Catechin Equvalent / g dry weight; mg GAE/g: mg Gallic Acid Equavilant / g dry weight

variety yielded 18 identified constituents, accounting for 96.87% of the sample. The main predominant compounds in ginger oil were geranial (32.75%), α-zingiberene (14.35%), γ-eudesmol (15.95%) and *cis*-sabinene hydrate (7.85%). The presence of geranial possibly contributed to the strong aroma reminiscent of lemon in the rhizomes similar to those of ginger from Australia [15]. Malek *et al*. [16] found high content of monoterpenoids (64.6%) in ginger volatile oil. They identified only 19 constituents and found much lower levels of camphene (1.8%) and geranyl acetate (8.8%). They did not detect geraniol but much higher levels of neral (14.2%), geranial (28.4%) and β-sesquiphellandrene (9.9%). As shown in table 1, Twenty-two compounds were identified in fenugreek EO represents 96.57% of the total EO. The major compounds were linolenic acid (24.28%), linoleic acid (22.65%), oleic acid, (15.23%), hexadecanoic acid (9.2%), octadecanoic acid (6.34%) and sotolon (3.28%).These results are in agreement with that of Mebazaa *et al*. [17] who reported that sotolon is known to be a key odourant compound of fenugreek seeds. Also, hexadecanoic acid (43.0%) and octadecanoic acid (2.44%) were identified in fenugreek EO.

Total Flavonoid and Phenolic Contents: The total flavonoid content (TFC) of spices was illustrated in Table 2. Ethanol was a better extraction solvent of TFC when compared to methanol. The highest content of TFC was noted in ethanol extracts of cinnamon and marjoram (78.3 and 68.12 mg catechin/g, respectively) while the lowest ones (25.83 mg catechin/g) was observed for methanol extract of fenugreek. In this case, the use of ethanol for extraction is recommended. The total phenol content (TPC) of cinnamon, fenugreek, marjoram and ginger are presented in Table 2. In the ethanol extract of cinnamon, a high content of total phenols (284.9 ± 6.8 mg GAE/g) was obtained in comparison with other extracts of marjoram, fenugreek and ginger. Ma1gorzata *et al*. [18] mentioned that cinnamon contain TPC of 172 mg GAE/g. Fenugreek is not a rich source of total phenols with (89.42 mg GAE/g) in ethanolic extract, which is very far from the total phenolic content in other ethanolic extract of marjoram and ginger (256 and 231 mg GAE/g), respectively. The TPC could be used as an important indicator of the antioxidant and antibacterial capacities due to their high redox potential allowing them to act as radical scavengers or hydrogen donors. In this study TPC of spices were positively correlated with TFC.

Antioxidant Activity by Diphenyl Picryl Hydrazyl (DPPH) Assay: The results of the radical scavenger assay for Egyptian spices extracts are presented in (Table 2 and Fig. 1). Cinnamon EO, ethanol and methanol extracts showed excellent radical scavenging activity, with inhibition % (I %) values of 89.6±5.23%, 83.1±4.07% and 75.1±5.02% at 200 µg/ml, respectively. The free radical scavenging activity of volatile oil was superior when compared with the ethanol and methanol extracts. The most effective essential EO appear to be those containing eugenol (clove and cinnamon), in agreement with the concept that the structural feature required for a strong free radical-scavenging activity is a phenolic group containing an electron repelling group in the O-position to the phenolic group. Vαgi *et al*. [19] reported the Egyptian marjoram EO and its solvent extracts possessed high antioxidant activities. The antioxidant activity of marjoram EO may be attributed to

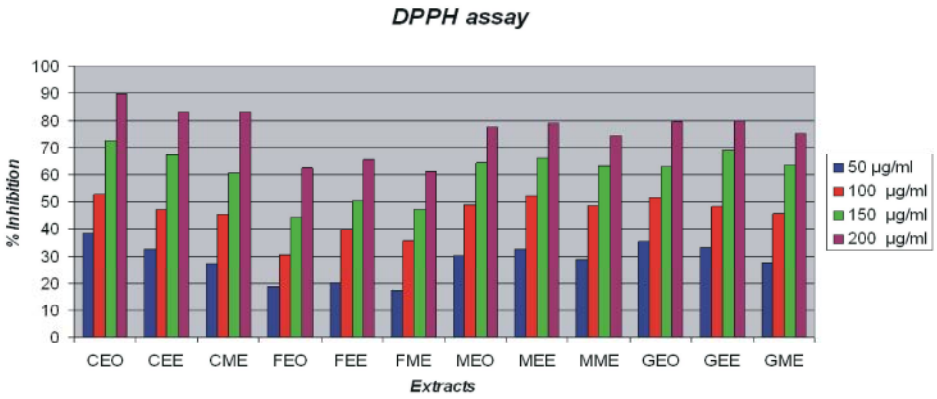

Fig. 1: Antioxidant activity at different concentrations of cinnamon, fenugreek, marjoram and ginger EOs, ethanol and methanol extracts by DPPH assay.

oxygenated terpenes as terpinene-4-ol (36.6%), linalool (11.6%) and cis-thujanol (5.13%), also, ethanol extract has higher I% than methanol extract with 79.2±7.0% and 74.6±4.06%, respectively. Ginger EO, ethanolic and methanolic extracts exhibited a highly antioxidant activity with 79.4±5.7%, 80.1±3.9% and 75.3±4.8%, respectively. Our results are found in agreement with Rehman *et al*. [20] who found ginger EO and its solvent extracts possess good thermal stability and exhibited 85.2% inhibition of peroxidation of linoleic acid when heated at 185°C for 120min. Therefore the use of ginger EO and its extracts is recommended as a natural antioxidant to suppress lipid oxidation in processed food.

ABTS Scavenging Assay: Table 2 shows the ABTS radical-scavenging activity of the cinnamon, fenugreek, marjoram and ginger. It can be seen that EOs presents different scavenging capacities. A concentration-dependent scavenging activity was found for all the EOs studied. The EOs and ethanol extracts from cinnamon, ginger and marjoram showed the highest ABTS scavenging capacity. The values ranging between of 1023±51.3 to 778.4 ± 33.5 µmol trolox /g. These results were in agreement with those reported by Ma1gorzata *et al*. [18], where the cinnamon and ginger EOs record 1119.9 and 839.4 µmol Trolox/g, respectively. Ginger is one of the most widely used herbs that contains several interesting bioactive constituents and possesses health promoting properties.

Antifungal Activity of Four Essential Oils: The *in vitro* antifungal activity of the essential oils (Eos) from four plants were tested against three food spoilage and mycotoxin producing fungi. They were analyzed quantitatively and recorded as inhibition of fungal mycelial growth and conidial germination according to the presence or absence of inhibition zones (Table 3). The antifungal activities of the tested EOs against fungi may be due to the presence of antimicrobial compounds in Eos [5]. Cinnamon EO showed an inhibitory capacity, at all the concentrations ($p < 0.01$) against *F. moniliforme* with inhibition zones ranging between 0.0 and 13.08 mm, *A. flavus* with inhibition zones between 0.0 and 19.54 mm and *A. ochraceus* with inhibition zones ranging between 0.0 and 15.76 mm. Total inhibition of conidial germination of these fungi was observed at 400 and 800 ppm, respectively. On the other hand fenugreek EO showed inhibition zones for *F. moniliforme* with inhibition zones ranging between 0.0 and 20.5 mm, *A. flavus* with inhibition zones between 0.0 and 29.5 mm and *Aspergillus ochraceus* with inhibition zones ranging between 0.0 and 26.3 mm. Total inhibition of conidial germination of *F. moniliforme* was reported at 400 ppm. *A. flavus* and *A. ochraceus* was reported at 800 ppm. The highest reductions (13.08-19.54 log CFU mL-1) for *F. moniliforme* and *A. flavus* were achieved with cinnamon. Our results are in agreement with that of Matan *et al*.[21], who worked with strains of A. *flavus* and *F. moniliforme*, respectively. The other two oils at one or more tested concentrations showed some activity against one or more micro-organisms. Moderate activity at concentration 800 ppm was observed for the ginger and marjoram EOs. Ginger EO at 800 ppm , total inhibition was not obtained for all tested fungal strains (Table 3) while, the growth of *F. moniliforme* was totally inhibited at concentration of 800 ppm of marjoram EO. Several studies showed that, ginger and marjoram EOs have an inhibitory effect on the growth of *A. flavus* and *A. ochraceus* [14-16].

Table 3: Effect of essential oils on the growth of the tested fungi

		Mean diameter±SD of mycelial growth in mm		
		Fungal species		
Essential oils	Conc. (ppm)	*Fusarium moniliforme*	*Aspergillus flavus*	*Aspergillus ochraceus*
CEO	100	13.08±0.31	19.54±0.93	15.76±2.41
	200	2.84±0.11	6.03±0.52	6.17±0.5
	400	0.0±0.0	4.86±0.13	3.01±0.22
	800	-	0.0±0.0	0.0±0.0
FEO	100	20.5±1.3	29.5±1.9	26.3±2.1
	200	9.12±0.34	19.8±2.1	21.4±1.8
	400	3.0±0.27	11.3±0.35	3.45±0.11
	800	0.0±0.0	0.0±0.0	0.0±0.0
GEO	100	48.45±2.4	55.1±2.2	47.2±2.3
	200	36.5±2.7	38.10±1.6	40.15±1.9
	400	23.15±1.9	32.4±1.6	13.2±0.8
	800	3.5±0.19	8.4±0.34	6.0±0.2
MEO	100	52.14±2.4	61.13±2.4	55.21±2.1
	200	41.23±2.1	52.40±1.7	46.18±1.9
	400	11.4±0.14	16.50±0.33	13.50±0.76
	800	0.0±0.0	6.02±0.14	3.45±0.27
Control	0	89.0±3.2	89.0±3.2	89.0±3.2

(-): Not detected; CEO: Cinnamon essential oil; FEO: fenugreek essential oil; MEO: marjoram essential oil and GEO: ginger essential oil; Data is average of four recordings from two separate experiments.

Antibacterial Activity of EOs: As regards antibacterial activity, the results summarized in Table 4 revealed that the selected essential oils showed antibacterial activity against six bacterial strains with varying magnitudes. The zone of inhibition above 7 mm in diameter was taken as positive result. Cinnamon and fenugreek EOs were the most effective as an antibacterial agent and showed maximum activity against all the six bacterial species tested. On the other hand, there was no inhibition of growth with the vehicle control (10% DMSO). Moderate effects were seen in ginger EO. Both gram-positive and gram-negative bacteria were sensitive to the potent essential oils. Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope [22]. The observed resistance of *E. coli* probably could be due to cell membrane permeability or due to other genetic factors. Among the six tested micro-organisms, *S. aureus* was, in general, the most susceptible microbe to most EOs studied. These results are in the same trend with those observed by El-Astal *et al*.[22] which were carried on some medicinal plant EOs.

Table 4: Antibacterial activity of essential oils against gram-positive and gram-negative bacteria using disc diffusion method

		Inhibition zone (mm) ± S.D of different bacterial strains					
		Gram - Positive bacteria strains			Gram-Negative bacteria strains		
Essential oils	Conc. (ppm)	*Bacillus subtilis*	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Escherchia coli*	*Pseudomonas aeruginosa*
CEO	01:01	27.34 ± 0.41	31.64 ± 0.89	28.42 ± 0.41	19.76±1.5	28.71±1.2	25.32±1.21
	01:05	24.57 ± 0.71	29.3 ± 1.32	23.4 ± 1.32	17.77±0.9	26.14±0.94	21.63±0.77
	01:10	16.76 ±0.96	22.21 ± 1.56	22.13 ± 0.92	14.06±1.2	22.88±2.5	19.02±1.31
	01:20	13.25 ±0.68	18.9± 0.54	19.12 ± 0.93	12.75±0.36	21.24±1.3	17.11±0.36
FEO	01:01	22.23±2.47	24.01±4.53	23.24±2.11	18.3±1.03	26.53±2.3	23.15±0.17
	01:05	18.11±0.61	22.25±1.22	21.56±2.51	15.42±0.41	24.86±2.1	19.6±0.82
	01:10	10.03±2.65	22.12±3.62	18.25±1.44	13.15±0.33	20.67±0.42	19.51±1.33
	01:20	8.33±1.11	19.35±3.15	13.89±1.84	11.22±0.91	19.38±2.4	13.20±0.54
GEO	01:01	16.86±2.47	16.22±1.91	15.87±0.23	13.25±1.5	18.76±0.5	15.85±1.92
	01:05	16.15±0.51	14.30±0.36	13.45±0.14	12.45±0.8	16.25±1.1	13.46±1.41
	01:10	10.02±0.52	11.5±0.52	13.25±0.25	10.68±2.1	13.05±2.0	10.25±0.47
	01:20	6.89±0.13	10.38±0.41	10.47±0.32	10.25±0.7	10.98±0.8	10.11±0.54
MEO	01:01	13.25±2.71	14.61±1.52	18.50±2.31	13.87±2.4	16.10±1.2	13.24±0.92
	01:05	11.20±1.51	12.47±2.22	16.35±0.83	13.25±0.51	13.45±0.8	11.94±1.21
	01:10	6.25±0.31	10.83±0.16	11.89±1.12	12.85±0.3	10.84±0.5	11.16±0.34
	01:20	6.10±0.27	10.50±0.5	9.25±0.87	10.64±0.6	10.25±0.3	11.10±0.22
Control	01:01	--	---	--	--	--	--
Gentamycin*	01:01	16.65 ± 0.42	14.78 ± 0.13	19.08 ± 0.24	19.64±1.51	25.02±0.83	19.22±1.55

CEO: Cinnamon essential oil; FEO: fenugreek essential oil; MEO: marjoram essential oil and GEO: ginger essential oil; * Gentamycin disc (25 µg) as a positive reference standard; Values are mean inhibition zone (mm) ± S.D of three replicates. (-) = no activity.

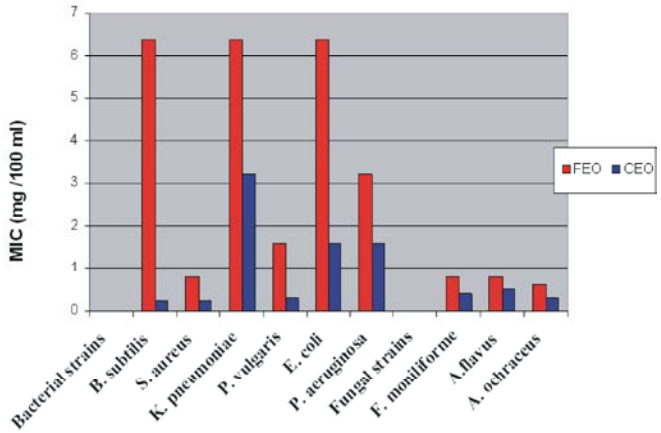

Fig. 2: Minimum Inhibitory Concentration (MIC) of the most active essential oils (fenugreek oil and cinnamon oil) of different bacterial and fungal strains.
FEO: fenugreek essential oil and CEO: Cinnamon essential oil

The antibacterial activity of cinnamon oil was probably due to their major component, cinnam aldehyde and their properties could be multiple. Cinnamon EO was not harmful when consumed in food products and it inhibited the growth of molds, yeast and bacteria, cinnamon oil had strong and consistent inhibitory effects against various pathogens [21]. Also, Farooqi and Sreeramu [23] revealed that the leaves of marjoram have antimicrobial activity against *Bacillus anthracis*, *Proteus vulgaris*, *Streptococcus agalactiae* and *Aspergillus fumigatus*.

Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentration (MIC) of EOs is presented in (Fig. 2). The obtained data revealed that cinnamon oil showed maximum activity with MIC values ranging from 0.25 to 3.2 mg/100ml followed by fenugreek oil with MIC values ranging from 0.8 to 6.4 mg/100ml against all the tested microorganisms strains. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Although the EOs showed antimicrobial activity, the reason behind this capacity is not well documented. This antimicrobial activity could be provoked by the major compounds of the EOs or due to a synergistic effect between the major compounds and the minor ones. The antimicrobial mechanisms may be an attack on the cell membrane's phospholipid bilayer, the disruption of the enzymatic systems, the formation of fatty acid hydroperoxidase by the oxygenation of unsaturated fatty acids [24, 25].

CONCOLUSION

Egyptian cinnamon, fenugreek, marjoram and ginger extracts, especially essential oils could be considered good sources of natural compounds with antioxidant and antimicrobial activities, mainly antibacterial. This can be attributed to the high concentration of the bioactive constituents or to the synergy with other different oil constituents. These essential oils could be applied, as additives, in food, cosmetic and pharmaceutical industries.

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