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(Original Article)

Chemical Composition and Antimicrobial Activity of Anise and Fennel Essential Oils

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Abstract

This study aims to knowledge the chemical composition of essential oils (EOs) extracted by hydro steam distillation of anise (*Pimpinella Anisum*) and fennel (*Foeniculum vulgare*) seeds which analyzed by gas chromatography-mass spectrometry (GC-MS). Whereas the main components of anise essential oil (AEO) were Transe Anethole (91.75%), γ-Himachalene (2.28%) and Carvone (1.71%). While the main constituents of fennel essential oil (FEO) were Estragole (74.46%), Transe Anethole (10.38%), D-limonene (10.06%) and Fenchone (3.04%). The antimicrobial activities of AEO and FEO (inhibition zone in mm) were evaluated, the results indicated that, AEO and FEO showed inhibition zones against *Bacillus cereus* (8 and 8 mm), *Bacillus subtilis* (10 and 10 mm), *Staphylococcus aureus* (13 and 13 mm), *Escherichia coli* (12 and 8 mm), *Pseudomonas aeruginosa* (8 and 9 mm) and *Salmonella typhimurium* (10 and 9 mm), *Aspergillus flavus* (24 and 18 mm), *Aspergillus niger* (13 and 17 mm), *Cladosporium sphaerospermm* (14 and 14 mm), *Mucor racemosus* (18 and 15 mm), *Penicillium chrysogenum* (22 and 33 mm), *Rhizopus arrhizus* (26 and 24 mm), *Candida albicans* (18 and 28 mm), *Debaryomyces hansenii* (12 and 10 mm) and *Pichia membranifaciens* (26 and 12 mm) for AEO and FEO; respectively. It was concluded from this study that the essential oils extracted from fennel and anise show antimicrobial activity against many harmful microorganisms.

***Keywords**: GC/MS, Essential oil, Anise; Fennel, Antimicrobial activity.*

Introduction

Essential oils (EOs) and their ingredients are widely used as a source of flavor in food making. Also, it has antioxidant and antimicrobial characteristics. Some studies showed the components responsible for the antimicrobial in these oils (Sanchez *et al*., 2011). The EOs that contains a high level of phenolic components has antimicrobial effects against common pathogenic bacteria (Lambert *et al*., 2001). These compounds can dissolve the outer membrane of Gram-negative bacteria and increase the permeability of the cytoplasmic membrane of the bacterial cell.

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In addition, aromatic plants are used in the kitchen and food industry in dry and fresh forms as spices or seasonings. The quality of aromatic spices depends on the content and chemical composition of the EOs (Lis *et al*., 2007). Many herbs and spices are potential sources of natural antioxidants. EOs as a product of secondary plant metabolism plays important roles in plant protection such as antiviral, antibacterial, antifungal and insecticidal properties against herbivores. Most of them are used as spices and medicines (Chouham *et al*., 2017). According to Bakkali *et al*. (2008) of about 3,000 EOs which are currently known, approximately 300 of them are commercially important in the food, sanitation, pharmaceutical, agronomic, cosmetics, and perfume industries. Anise (*Pimpinella anisum*) and fennel (*Foeniculum vulgare*) a plant belonging to Apiaceae family, which grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain (Downie *et al*., 2010and Salehi Surmaghi., 2010). Saber and Eshra (2019) showed that the proximate composition of fennel seeds contains moisture, crude protein, crude fat, crude fiber and ash as 8.04, 10.18, 10.71, 18.01 and 12.87%; respectively and the total carbohydrate was 40.19% (by difference). Khammassi *et al*. (2018) observed that the EOs yields from edible fennels ranged from 1.2 to 5.06%. While anise seed contains 1.5- 6.0 % of EOs and 8-11% of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 % carbohydrates and 18 % protein (Besharati-Seidani *et al*., 2005).

Khubeiz and Zahraa (2020) found that the EOs yield of anise seeds was 2.93%, the analysis of anise essential oil (AEO) by GC-MS showed that five components were identified, accounting for 99.09 %,which were trans anethole 96.11%, γ-himachalene 1.83%, α-zingiberene 0.53%, α-Cadinol 0.33% and estragole 0.29%.

Ahmed *et al*. (2019) found that GC–MS analysis of Egyptian FEO identified 27 constituents. The major constituents of them were estragole (51.04%), limonene (11.45%), l-fenchone (8.19%) and trans-anethole (3.62%). For FEO from Chinese GC–MS analysis identified 30 constituents, the major constituents were trans-anethole (54.26%), estragole (20.25%), l-fenchone (7.36%) and limonene (2.41%). The chemical composition of FEO from two countries was very different. Shahat *et al*. (2011) found that the main components of FEO were estragole (57.94%), limonene (20.64%). fenchone (7.22%) and trans-anethole (4.99%). Also, Starovic *et al*. (2016) found 31 compounds in fennel essential oil by GC–MS analysis , and the main constituent was estragole (methyl chavicol) (61.75 %) followed by fenchone (25.66 %).

Amer and Aly (2019) showed the antibacterial effects of AEO, the largest inhibition zones were 21.0, 18.3, 9.7 and 7.0 mm for *Bacillus cereus*, *Staphylococcus aurous*, *Salmonella typhimurium*, and *Escherichia coli*; respectively. Antimicrobial activities of anise extracts and AEO may be attributed to their phenolic contents since numerous phytochemical studies indicated the presence of noticeable amounts of phenolic compounds in anise. On the same trend, Also Saber and Eshra (2019) found the fennel seeds and the oil

extract have antibacterial activity against Gram negative and Gram-positive bacteria; the higher effect was found on Gram-positive bacteria (*Staphylococcus aureus*), than Gram-negative bacteria (*Escherichia coli*). While the oil extract was more effective than the seed extract in the diffusion assay (inhibition zone in mm).

Materials and Methods

Aromatic plant samples

Plant samples of two aromatic plants used in this study namely: Anise (*Pimpinella anisum* L.) and Fennel (*Foeniculum vulgare*) were obtained from a National Research Centre, Cairo, Egypt.

Chemicals: All chemicals used (analytical grades) were purchased from Sigma (St. Louis, USA) and from EL-Gamhouria for Trading Chemicals and Drugs Company, Assiut, Egypt.

Essential oils (EOs) extraction

The essential oils (EOs) of two selected aromatic plants (anise and fennel seeds) were extracted by hydro steam distillation using the Clevenger apparatus according to the method described in the British Pharmacopeia (1988). The EOs was collected after 4 hours. The separated EOs was dried over Na_2SO_4 (anhydrous) before hold in dark glass bottles at 4°C until analysis.

Chemical composition of aromatic plants

Moisture content, crude protein, crude fat, crude fiber and ash contents of aromatic plant samples were estimated as described by AOAC (2010).

Physicochemical properties of EOs

Specific gravity, peroxide value (PV) and acid value (AV) were demonstrated according to AOCS (1998). Refractive index (RI) was determined by an Abbe 60 refractometer at 20°C (AOAC, 2010).

Gas chromatography–mass spectrometry analysis (GC-MS) of AEO and FEO

The essential oils were analyzed by GC/MS according to the method described by Adams (1989).The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples (AEO and FEO) were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using hydrogen as carrier gas at a flow rate of 1.0 ml/min at a split 1:20 of, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4°C /min to 150°C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280°C and 220 °C; respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 4 min. Identification of different constituents was

determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Antimicrobial activity of AEO and FEO

Growth conditions of microbial strains

The antimicrobial activity both of AEO and FEO was screened on different species of bacteria, fungi and yeasts. The antibacterial activity was tested against 6 bacterial strains; three Gram-positive bacteria: *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* and three Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Antifungal activities were tested using six fungal strains; *Aspergillus flavus*, *A. niger*, *Cladosporium sphaerospermum*, *Mucor racemosus*, *Penicillium chrysogenum* and *Rhizopus arrhizus*. The anti-yeast activities were determined against three yeast strains: *Candida albicans, Debaryomyces hansenii* and *Pichia membranifaciens*. Suspensions with 0.5 McFarland turbidity were prepared from the pure cultures in Nutrient broth for bacteria and sabouraud dextrose broth for fungi and yeasts by mixing the bacterial or fungal cells from fresh cultures. Bacteria, fungi and yeasts strains obtained from Assiut University Mycological Center (AUMC).

Determination of antimicrobial activity

Agar well diffusion method according to the NCCLS (1993) was used for screening the antibacterial and antifungal activities of AEO and FEO. 1 ml of freshly prepared bacterial or fungal suspensions was pipette onto sterile Petri dishes. Molten Nutrient agar (NA) for bacteria and Sabouraud dextrose agar (SDA) for fungi and yeasts were poured into the Petri dish containing the inoculum and mixed well. After solidification, wells were made using a sterile cork borer (5 mm in diameter) into agar plates. Then, 50 µl of each oil was added to the wells. Then, the plates were incubated at 37^{o}C/48 hours for bacteria and at 28^{o}C/5-7days for fungi and yeasts, all tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring the zone of inhibition (including the diameter of the wells) that appeared after the incubation period.

Results and Discussion

Chemical composition of aromatic plants

The chemical contents of anise and fennel seed samples are shown in Table 1. Given data illustrated that all tested aromatic plant specimens consist of moisture, crude protein, crude fat, crude fiber and ash diverse between (6.11 and 5.88%), (17.56 and 12.72%), (13.56 and 10.16%), (16.86 and 14.33%) and (6.15 and 9.22%) of anise and fennel seeds sequence. From these results it could be conclude that aromatic plant samples have a crucial and worthy nutritional and industrial value. These results are in the same direction with those which obtained by Besharati-Seidani *et al*. (2005) and Saber and Eshra (2019).

Table 1. Chemical composition content of anise and fennel seeds (dry weight basis %)

Chemical composition	Anise seeds	Fennel seeds
Moisture (%)	6.11	5.88
Crude protein (%)	17.56	12.72
Crude fat (%)	13.56	10.16
Crude Fiber (%)	16.86	14.33
Ash (%)	6.15	9.22

All data presented in this table are a mean of 3 replicates.

Essential oil Content of aromatic plant samples

The essential oil Content of the studied aromatic plants is depicted in Table 2. Illustrated data show that quantities values of essential oil that extracted from anise and fennel seeds by hydro steam distillation (Clevenger apparatus) were 2.86 and 3.21%; respectively. These results are agreement with that of Shojaii and Abdollahi (2012) and Khammassi *et al*. (2018).

Table 2. Essential oils (EOs) Content of aromatic plants

Aromatic plant varieties	Essential oil (%)
Anise seeds	2.86
Fennel seeds	3.21

Extraction of EOs was done in anise and fennel seeds (in dry weight). All data presented in this table are a mean of 3 replicates.

The physical and chemical characteristics of AEO and FEO

The physical and chemical characteristics of EOs are proved that there is a huge ability to use these oils in applications of nutrition and industry. Characteristics of AEO and FEO are shown in Table (3). Refractive index and acid value were higher in AEO (1.5423 and 1.68) compared to FEO (1.5114 and 1.18); respectively. Peroxide value was used as an index of the degree of oxidative rancidity of oils. The peroxide value (meq/kg) was lower in FEO (0.66), compared with that in AEO (0.72). These results are somewhat agreed with those reported by El-Kashef (2014), Salim *et al*. (2016) and Sun *et al*. (2019). The given results depicts that there were differences in refractive index, specific gravity, color, acid value and peroxide value between the extracted EOs from anise and fennel seeds.

Table 3. Characteristics of AEO and FEO

Essential oil characteristics	AEO	FEO
Refractive index at 20ºC	1.5423	1.5114
Specific gravity (g/cm3) at 20ºC	0.987	0.962
Acid value(mg KOH/g)	1.68	1.18
Peroxide value(meq/kg)	0.72	0.66
Color	pale yellow	Yellow

AEO = Anise essential oil FEO= Fennel essential oil

All data presented in this table are a mean of 3 replicates.

Chemical composition of AEO and FEO

The chemical composition of AEO was fractionated by GC/MS as shown in Table (4) and Fig. (1). The obtained results revealed that, the isolated and identified fractions of AEO were 34 components forming 99.98% of total essential oil. The major components of AEO were Transe Anethole (91.75%) followed by γ-Himachalene (2.28%), Carvone (1.71%), Estragole (Methyl chavicol) (0.66%), Trans-Pseudoisoeugenyl-2-methylbutyrate (0.46%), α-Longipinene (0.32%), β-Bisabolene (0.32%), Sabinene Hydrate (0.26%), p-Isopropyl benzaldehyde (0.23%), Linalool (0.21%), Humulene (0.19%), Butanoic acid, 2-methyl-, 4-methoxy-2-(3-methyloxiranyl) phenyl ester (0.18%), α-Curcumene (0.17%), D-Limonene (0.15%), γ-Terpinene (0.14%), γ-Muurolene (0.12%) and others compounds were found in small quantities in AEO less than 0.1%, these results agreed with Bettaieb Rebey *et al*. (2018) .

On the other side the results in Table (5) and Fig. (2) showed that GC/MS analysis of FEO recorded 28 constituents. these compounds form 99.96% of the total FEO, The constituents of the FEO were Estragole (Methyl chavicol) (74.46%), Trans Anethole (10.38%), D-limonene (10.06%), Fenchone (3.04%), γ-Terpinene (0.35%), Ortho-Cymene (0.32%), p-Isopropylbenzaldehyde (0.31%), Cis-Ocimene (0.14%), Cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl (1H)benzocyclo heptene (0.14%), 2-Isopropylidene-3-methylhexa-3,5-dienal (0.12%), β-Myrcene (0.11%) and others compounds were found in the FEO less than 0.1% (pity amounts). The composition map resulted from the present study is similar to that recorded by Khammassi *et al*. (2018) they found that estragole (methyl chavicol) (66.09–85.23%), fenchone (5.18–23.09%) and limonene (4.3–10.25%) to be the major compounds that identified in FEO. On the other hand, Telci *et al*. (2009) differ by finding a higher value for trans-anethole (84.12%) and lower for estragole (4.19-5.53%), limonene (2.96-4.69%) and fenchone (1.17-2.65%) of FEO. These differences in the components and their content of fennel essential oil may be due to the differences in the regions source of cultivated types and maturity of fennel seeds. In addition to this, extraction methods and analysis conditions of EOs can have a controversial effect (Díaz-Maroto *et al*., 2006).

Table 4. Chemical composition of anise essential oil (AEO) by GC/MS

Peak No.	RT*	Compound	Formula	% Area
1	4.116	2-Methylbutanoic acid	$C_5H_{10}O_2$	0.02
2	6.096	β-Ocimene	$C_{10}H_{16}$	0.09
3	7.337	α-Terpinolene	$C_{10}H_{16}$	0.04
4	7.60	β-Cymene	$C_{10}H_{14}$	0.09
5	7.703	D-Limonene	$C_{10}H_{16}$	0.15
6	8.693	γ-Terpinene	$C_{10}H_{16}$	0.14
7	10.244	Linalool	$C_{10}H_{18}O$	0.21
8	12.63	Sabinene Hydrate	$C_{10}H_{18}O$	0.26
9	13.202	α-Terpineol	$C_{10}H_{18}O$	0.07
10	13.425	Estragole (Methyl chavicol)	$C_{10}H_{12}O$	0.66
11	14.776	p-Isopropyl benzaldehyde	$C_{10}H_{12}O$	0.23
12	14.894	Carvone	$C_{10}H_{14}O$	1.71
13	15.817	p-Anisaldehyde	$C_8H_8O_2$	0.07
14	16.698	Trans Anethole	$C_{10}H_{12}O$	91.75
15	18.695	α-Copaene	$C_{15}H_{24}$	0.01
16	19.148	Bicyclo[2.2.2]octa-2,5-diene, 1,2,3,6-tetramethyl-	$C_{12}H_{18}$	0.10
17	20.034	Trans-Caryophyllene	$C_{15}H_{24}$	0.03
18	20.475	β-Longipinene	$C_{15}H_{24}$	0.01
19	21.15	Humulene	$C_{15}H_{24}$	0.19
20	21.333	Aromandendrene	$C_{15}H_{24}$	0.03
21	22.089	γ-Himachalene	$C_{15}H_{24}$	2.28
22	22.186	γ-Muurolene	$C_{15}H_{24}$	0.12
23	22.398	α-Curcumene	$C_{15}H_{22}$	0.17
24	22.598	Valencene	$C_{15}H_{24}$	0.02
25	22.775	α-Longipinene	$C_{15}H_{24}$	0.32
26	23.016	Eugenol Methyl	$C_{11}H_{14}O_2$	0.07
27	23.17	β-Bisabolene	$C_{15}H_{24}$	0.32
28	23.325	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	$C_{15}H_{24}O$	0.02
29	23.588	Bicyclo[4.1.0]heptan-2-ol, 1.beta.-(3-methyl-1,3-butadienyl)-2.alpha.,6.beta.-dimethyl-3.beta.-acetoxy-	$C_{16}H_{24}O_3$	0.06
30	24.046	α-Calacorene	$C_{15}H_{20}$	0.03
31	26.678	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2 -methylenebicyclo[4.1.0]heptane	$C_{15}H_{22}$	0.04
32	29.213	β-Cedrene	$C_{15}H_{24}$	0.03
33	33.790	Trans-Pseudoisoeugenyl-2-methylbutyrate	$C_{15}H_{20}O_3$	0.46
34	36.199	Butanoic acid, 2-methyl-, 4-methoxy-2-(3-methyloxiranyl)phenyl ester	$C_{15}H_{20}O_4$	0.18
		Total		**99.98 %**

*RT= Retention time

Table 5. Chemical composition of fennel essential oil (FEO) by GC/MS

Peak No.	RT*	Compound	Formula	% Area
1	4.236	β-Myrcene	$C_{10}H_{16}$	0.11
2	4.671	α-Phellandrene	$C_{10}H_{16}$	0.04
3	5.146	α-Terpinene	$C_{10}H_{16}$	0.02
4	5.455	Ortho-Cymene	$C_{10}H_{14}$	0.32
5	5.626	D-Limonene	$C_{10}H_{16}$	10.06
6	6.061	Cis-Ocimene	$C_{10}H_{16}$	0.14
7	6.473	Santolina triene	$C_{10}H_{16}$	0.02
8	6.811	γ-Terpinene	$C_{10}H_{16}$	0.35
9	7.921	Fenchone	$C_{10}H_{16}O$	3.04
10	8.682	β-Pinene	$C_{10}H_{16}$	0.08
11	9.437	Z-Citral	$C_{10}H_{16}O$	0.04
12	9.843	Perilla Alcohol	$C_{10}H_{16}O$	0.01
13	11.623	Allo Ocimene	$C_{10}H_{16}$	0.07
14	12.865	Estragole (Methyl chavicol)	$C_{10}H_{12}O$	74.46
15	14.043	P-Isopropylbenzaldehyde	$C_{10}H_{12}O$	0.31
16	15.651	2-Isopropylidene-3-methylhexa-3,5-dienal	$C_{10}H_{14}O$	0.12
17	15.84	Trans Anethole	$C_{10}H_{12}O$	10.38
18	18.65	γ-Muurolene	$C_{15}H_{24}$	0.03
19	20.012	Trans-Caryophyllene	$C_{15}H_{24}$	0.04
20	21.093	α-Humulene	$C_{15}H_{24}$	0.01
21	21.86	Cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	$C_{15}H_{24}$	0.14
22	21.991	α-Amorphene	$C_{15}H_{24}$	0.05
23	22.638	α-Acoradiene	$C_{15}H_{24}$	0.03
24	23.038	Eugenol Methyl	$C_{11}H_{14}O_2$	0.03
25	23.376	Delta-Cadinene	$C_{15}H_{24}$	0.03
26	24.995	Caryophyllene Oxide	$C_{15}H_{24}O$	0.01
27	26.855	β-Acoradiene	$C_{15}H_{24}$	0.01
28	33.893	3-Methyl-2-(2-methylallyl)furan	$C_9H_{12}O$	0.01
		Total		**99.96 %**

*RT= Retention time

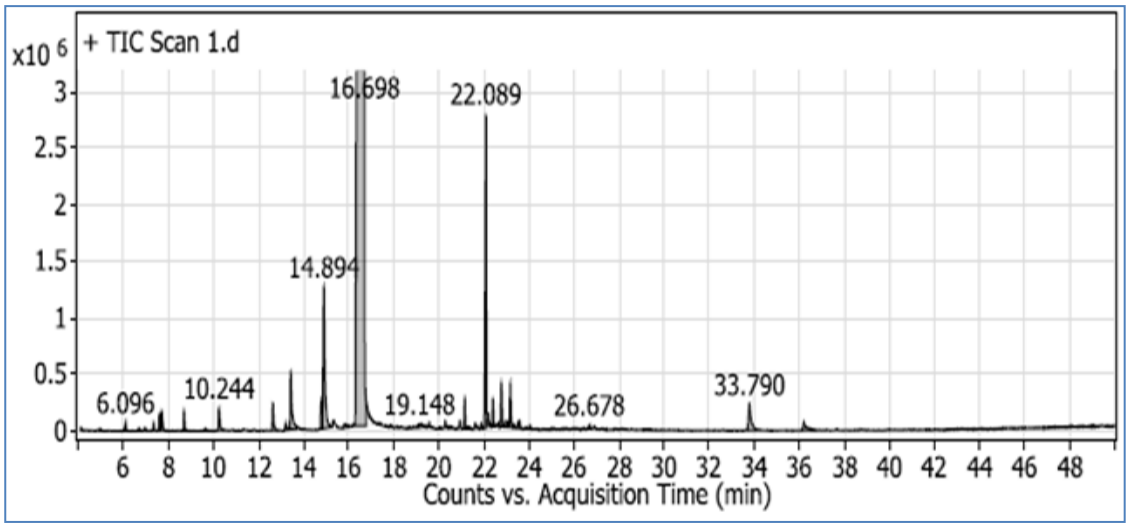

Fig. 1. GC/MS chromatogram of AEO.

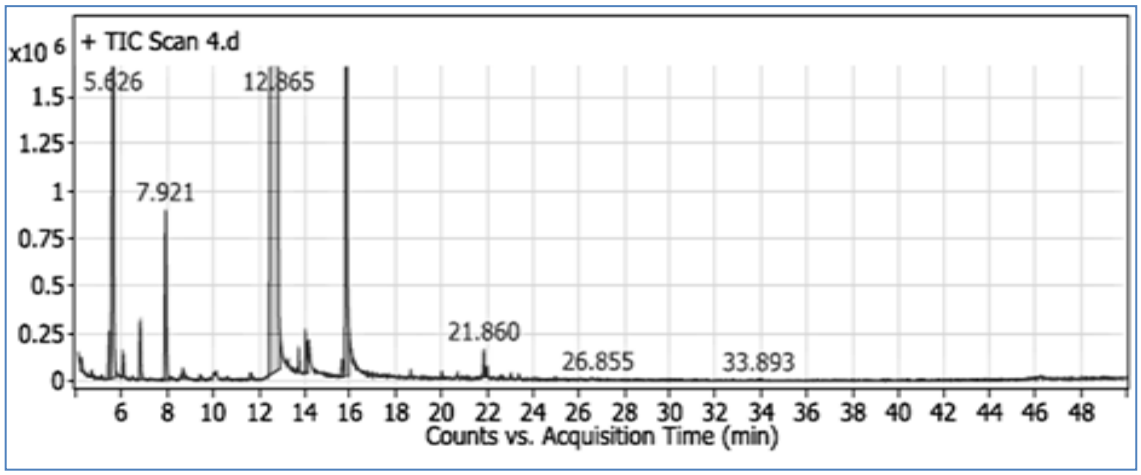

Fig. 2. GC/MS chromatogram of FEO.

Antimicrobial activity of AEO and FEO samples

Antimicrobial activity (inhibition zone in mm) of the EOs extracted from anise and fennel seeds are depicted in Tables 6 and 7. Chloramphenicol used as standard or positive control for the antibacterial activity and clotrimazole used as standard or positive control for the antifungal and anti-yeasts activity, Dimethyl sulfoxide (DMSO) (no addition EOs) was used as a negative control. The results in Tables 6 and 7 showed that, Gram-positive bacteria and Gram-negative were have a sensitivity to AEO and FEO specimens. In addition, EOs from anise and fennel were antifungal and anti-yeasts activity. Results showed that the antibacterial activity (inhibition zone in mm) were 8 & 8 mm for *Bacillus cereus*, 10 & 10 mm for *Bacillus subtilis*, 13 & 13 mm for *Staphylococcus aureus*, 12 & 8 mm for *Escherichia coli*, 8 & 9 mm for *Pseudomonas aeruginosa* and 10 & 9 mm for *Salmonella typhimurium* for AEO & FEO; respectively. On the other hand, antifungal activity (inhibition zone) was 24 & 18 mm for *Aspergillus flavus*, 13 & 17 mm for *Aspergillus niger*, 14 & 14 mm for *Cladosporium sphaerospermm*, 18 & 15 mm for *Mucor racemosus*, 22 & 33 mm for *Penicillium*

chrysogenum and 26 & 24 mm for *Rhizopus arrhizus* for AEO & FEO; respectively. While the anti-yeasts activity (inhibition zone) was 18 & 28 mm for *Candida albicans*, 12 & 10 mm for *Debaryomyces hansenii* and 26 & 12 mm for *Pichia membranifaciens* for AEO & FEO; respectively. These results are in agreement with those obtained by Diao *et al*. (2014) and Sun *et al*. (2019).

Table 6. Antibacterial activity (inhibition zone) of AEO and FEO samples

Bacterial Strains	inhibition zone (mm)			
	AEO	FEO	DMSO**	chloramphenicol*
***Bacillus cereus* (G +ve) AUMC No B-52**	8	8	-	20
***Bacillus subtilis* (G +ve) AUMC No. B-63**	10	10	-	17
***Staphylococcus aureus* (G +ve) AUMC No. B-54**	13	13	-	26
***Escherichia coli* (G -ve) AUMC No. B-53**	12	8	-	28
***Pseudomonas aeruginosa* (G -ve) AUMC No. B-73**	8	9	-	26
***Salmonella typhimurium* (G -ve) AUMC No. B-62**	10	9	-	23

AEO = Anise essential oil FEO = Fennel essential oil
*Chloramphenicol was used as positive control (standard) for the antibacterial activity
** Dimethyl Sulfoxide (DMSO) was used as negative control.
The amount added in each pore is 50 µl.
AUMC = Assiut University Mycological Center.
All data presented in this table are a mean of 3 replicates.

Table 7. Antifungal and anti-yeast activity (inhibition zone) of AEO and FEO samples:

Fungal Strains	inhibition zone (mm)			
	AEO	FEO	DMSO**	Clotrimazole*
***Aspergillus flavus* AUMC No. 1276**	24	18	-	24
***Aspergillus niger* AUMC No. 8941**	13	17	-	23
***Mucor racemosus* AUMC No. 5123**	18	15	-	18
***Cladosporium sphaerospermm* AUMC No. 14429**	14	14	-	20
***Penicillium chrysogenum* AUMC No. 9186**	22	33	-	28
Rhizopus arrhizus AUMC No. 4757	26	24	-	23
yeast Strains				
***Candida albicans* AUMC No. 1299**	18	28	-	24
***Debaryomyces hansenii* AUMC No. 2666**	12	10	-	14
***Pichia membranifaciens* AUMC No. 10760**	26	12	-	14

AEO = Anise essential oil FEO = Fennel essential oil
*Clotrimazole was used as positive control (standard) for the antifungal and anti-yeast activity.
** Dimethyl Sulfoxide (DMSO) was used as negative control.
The amount added in each pore is 50 µl.
AUMC = Assiut University Mycological Center.
All data presented in this table are a mean of 3 replicates.

It is clear from the obtained results that the microbial strains (bactria, fungi and yeasts) under study have sensitivity to AEO and FEO samples. Antibacterial, antifungal and anti-yeasts activity of EOs is due to the presence of certain secondary plant metabolites such as terpenoids, steroids and flavonoids, esters and acids which are identified in EOs (Upadhyay, 2015). Pramila *et al*. (2012) believed that the positive effects of EOs on microbial growth might be caused by their phenolic compounds altering the permeability of microbial cells when

interacting with membrane proteins. This in turn would lead to the deformation of cellular structure and function and subsequent loss of macromolecules from their interior. Also, antimicrobial activity of EOs differed with type, composition of plant, concentration of EOs, the tested microorganism, processing and storage conditions (Ozogul *et al*., 2015).

Conclusion

From this study it could be concluded that, AEO and FEO exhibit promising antimicrobial effects towards selected food harmful microorganism, which can be due to the presence of the bioactive constituents, such as terpenoids, steroids and flavonoids, esters and acids. These tested EOs and their main active components could be crucial candidates to be used as natural alternatives and can be applicable in food preservation to inhibit the bacteria and fungi growth and to extend the validity consuming of the food products.

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التركيب الكيميائي والنشاط المضاد للميكروبات لزيوت الينسون والشمر العطرية

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الملخص

تهدف هذه الدراسة الي معرفة التركيب الكيميائي للزيوت العطرية المستخرجة من بذور الينسون والشمر والتي تم تحليلها بواسطة الـ GC-MS ، كانت المكونات الرئيسية لزيت الينسون العطري هي الـ Transe Anethole (91.75%) و الـ γ-Himachalene (2.28%) والـ Carvone (1.71%). بينما كانت المكونات الرئيسية لزيت الشمر العطري هي الـ Estragole (74.46%) والـ Transe Anethole (10.38%) والـ D-limonene (10.06%) والـ Fenchone (3.04%). كذلك تم تقييم الأنشطة المضادة للميكروبات لزيت الينسون والشمر (منطقة التثبيط بالمليمتر) ، وأشارت النتائج إلى أن الزيوت العطرية أظهرت مناطق تثبيط بالمليمتر ضد *Bacillus cereus* (8 و8 ملم) ، *Bacillus subtilis* (10 و10ملم)، *Staphylococcus aureus* (13 و13 ملم)، *Escherichia coli* (12 و8 ملم)، *Pseudomonas aeruginosa* (8 و9ملم)، *Salmonella typhimurium* (10 و 9ملم)، *Aspergillus flavus* (24، 18 ملم)، *Aspergillus niger* (13 و 17ملم)، *Cladosporium sphaerospermm* (14، 14 ملم)، *Mucor racemosus* (18 و15ملم)، *Penicillium chrysogenum* (22 و33ملم)، *Rhizopus arrhizus* (26، 24ملم)، *Candida albicans* (18، 28 ملم)، *Debaryomyces hansenii* (12، 10ملم)، *Pichia membranifaciens* (26 ، 12 ملم) لكل من زيت الينسون وزيت الشمر علي التوالي. نستنتج من هذه الدراسة أن الزيوت العطرية المستخرجة من الينسون والشمر لها نشاط مضاد للميكروبات ضد العديد من الكائنات الحية الدقيقة الضارة.