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Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.)

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ABSTRACT

Essential oil and extracts of two Egyptian plants, fennel and chamomile were examined for their antioxidant and antimicrobial activities. The essential oil for fennel seeds and chamomile flowers were found to be 1.95 and 0.73%, respectively. Gas chromatography/mass spectrometry analysis of the essential oils revealed the presence of 15 major monoterpenoids in all two plant essential oil but their percentages in each plant were greatly different. *Trans*-anethole, estragole, fenchone and limonene were highly abundant in all of the examined oils. Antioxidant activities of the extracts were evaluated using the DPPH• radical scavenging. The statistical analysis showed that the highest antiradical power (ARP) was noticed for chamomile extracted by methanol, where is fennel extracted be hexane gave the least value which was 243. Antimicrobial activities of each plant extracts and essential oil were measured. The lowest MIC values of essential oils for *Aspergillus flavus*, *Candida albicans*, *Bacillus cereus*, and *Staphylococcus aureus* was obtained. The essential oils exhibit different degrees of antimicrobial activities depending on the doses applied. The results of the present investigation demonstrated significant variations in the antioxidant and antimicrobial activities of fennel and chamomile essential oil and extracts.

1. Introduction

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Because of the resistance that pathogens build against antibiotics, there is a growing interest to use natural antibacterial products for food preservation, like extracts of herbs and spices. Indeed, natural crude extracts and biologically active compounds from plant species used in traditional medicine may represent valuable sources for such new preservatives (Al-Fatimi et al., 2007).

In food processing, lipid oxidation not only causes a loss in nutritional and gustative quality of foods but also generates oxidized products such as free radicals which lead to various undesirable chemical reactions. To avoid or delay this autoxidation process, conventional artificial antioxidants such as bulylated hydroxyanisole (BHA) and bulylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) have been used for more than five decades. However, these synthetic antioxidants have been suspected to or promote negative health effects. For this reason, there is a growing interest in studies of natural additives as potential antioxidants. Many sources of antioxidants of plant origin have been studied in recent years. Among these, the antioxidant properties of many aromatic and medicinal plants have shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups (Kulisic et al., 2004). Therefore, the demands for these plants are increasing in industrialized and non-industrialized countries which lead to increasing their prices.

Recently, spices have received attention also in their useful physiological functions and antimicrobial activity. There are a lot of reports about antimicrobial activity of spice extracts and its essential oils. However, the available informations are for a small group of microorganisms and they are tested at high concentrations which are no practical use. More research is required on antimicrobial effects to food-related bacteria such as food spoilage bacteria and food borne bacterial pathogens (Liu and Nakano, 1996).

Fennel (*Foeniculum vulgare* L.) a plant belonging to the family Apiaceae, has a long history of herbal uses and widely cultivated, both in the native habitat, India and Egypt, and elsewhere, for its edible strongly flavored leaves and seeds. Parejo et al.

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(2002) evaluated thirty six different extracts of six herbs and aromatic plants (fennel, common melilot, milfoil, lavandin, spik lavender and tarragon) for their radicals scavenging activity by DPPH•, NBT/hypoxanthine superoxide, and •OH/luminol chemiluminescense methods, and for their antioxidant activity by the ß-carotene blenching test. They also determined the total phenolic content by the Folin–Ciocalteu method. The data obtained showed that, the distilled plant material was found to exhibit a higher phenolic content as well as antioxidant and radical scavenging activities than non-distilled material.

The antioxidant activity of water and ethanol extracts of fennel seeds was evaluated by various antioxidant methods, including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities and reducing power. Those various antioxidant activities were compared to standard antioxidants such as (BHA), (BHT), and ά-tocopherol. The water and ethanol extracts of fennel seeds showed strong antioxidant activity. Hundred μg of water and ethanol extracts exhibited 99.1% and 77.5% inhibition of peroxidation in linoleic acid system, respectively, and greater than the effect of the same dose of α-tocopherol (36.1%); (Oktay et al., 2003). Total phenolic compounds in fennel seeds extracts were determined as gallic acid equivalents.

Faudale et al. (2008) measured the antioxidant activity of Wild, medicinal and edible fennels as free radical (DPPH•), hydroxyl radical and superoxide anion scavenging activities. Wild fennel was found to exhibit a radical scavenging activity, as well as total phenolic and total flavonoids content, higher than those of both medicinal and edible fennels.

The antimicrobial activity of fennel essential oil was assessed by using disk diffusion method (Gulfraz et al., 2008). Fennel essential oil showed more inhibitory activity against *Bacillus cereus*, *Bacillus magaterium*, *Bacillus pumilus*, *Bacillus substilis*, *Eschericha coli*, *Klebsiella pneumonia*, *Micrococcus lutus*, *Pseudomonos pupida*, *Pseudomonos syringae*, and *Candida albicans* than the effect of methanolic and ethanolic fennel seed extracts. The lowest MIC values of fennel essential oil were noticed for *C. albicans* and *E. coli*. It was observed that essential oil and seed extracts of fennel exhibit different degree of antimicrobial activities depending on the doses applied. Therefore, fennel essential oil could be a source of pharmaceutical materials required for the preparation of new therapeutic and antimicrobial agents.

Chamomile (*Matricaria chamomilla* L.), family Compositae has been used in folk medicine throughout history. It is still used in the production of medicinal tea that is renowned for its calming properties. The blue essential oil of chamomile is used in the pharmaceutical cosmetic industries (Falzari and Menary, 2003).

Marino et al. (2001) tested essential oils from sage, mint, hyssop, chamomile, and oregano for their inhibitory effects against nine strains of gram-negative bacteria and six strains of gram-positive bacteria. The essential oils of sage, mint, hyssop and chamomile showed a bacteriostatic activity, besides; oregano essential oils appeared to be bactericidal at concentrations above 400 ppm, probably because of their high contents in phenolic compounds. The chemical analysis was unable to explain the antimicrobial effect. The bacteriostatic activity was more marked against gram-positive bacteria; in contrast, the bactericidal activity was greatest against gram negative bacteria.

The objectives of the present investigation were to evaluate the efficiency of different solvents in extracting the active principles from tested plants, and to elucidate their oxidative actions. To measure the antiradical activities of the tested aromatic plant extracts using the stable radical, 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH•) in comparison with some commercially available antioxidants. Also determine the antimicrobial activities of plant extracts and essential oils by using the disc-diffusion method and agar dilution method to find the minimum inhibitory concentration (MIC) *in vitro* of every plant extract.

2. Materials and methods

2.1. Collection of plant materials

Chamomile flowers (*M. chamomilla* L.) – Astraceae and fennel seeds (*F. vulgare* L.) – Apiaceae were randomly collected and stored in deep freeze at −20 °C until analysis.

2.2. Chemicals, solvents, reagents

2.2-Diphenyl-1-picrylhydrazyl (DPPH•), butylated hydroxy anisole (BHA), tertiary-butylated hydroquinone (TBHQ), ascorbic acid, α-tocopherol and Folin–Ciocalteu reagents were obtained from Sigma Chemical Co. Methanol and formic acid (HPLC grade) were purchased from Aldrich Co. All other solvents and chemicals were of analytical grade.

2.3. Preparation and extraction of the plant materials

Plant materials were ground and macerated for extraction. Twenty grams of each prepared sample were weighed into 1 l Erlenmeyer flasks, and then 200 ml of solvents with varying polarities (methanol, ethanol, diethyl ether and hexane) were added to the samples. Extraction was carried out by shaking at room temperature for 72 h. After filtration through filter paper (Whatman No. 4), the residue was re-extracted twice, and then the combined extracts of every sample were evaporated at room temperature and dried in desiccators under vacuum to a constant weight. The final residues were used to study their antioxidant activities (Pizzale et al., 2002).

2.4. Isolation of essential oil

Portions (100 g) of each prepared plant material were hydrodistilled for 6 h in a Clevenger type apparatus to isolate the essential oil. The obtained essential oils were dried over anhydrous sodium sulphate. After filtration they were stored in dark glass bottles at −4 °C until used for further analyses.

2.5. Microorganisms

Two species of Gram negative bacteria (*Escherichia coli* O157 ATCC 1659 and *Salmonella tyhpi* ATCC 13076) and two species of Gram positive bacteria (*B. cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 13565) and one species of yeast (*C. albicans* ATCC 10231) and one species of mold (*Aspergillus flavus* ATCC 16875) were used as indicator microorganisms for detection of the antimicrobial activity. All strains mentioned above were obtained as actively growing cultures from the microbial culture collection of the Microbiological Resources Center Cairo (MIRCEN), Faculty of Agriculture, Ain shams University, Cairo, Egypt.

2.6. Instrumentation

(a) All spectrophotometric data were acquired using JASCO V-530 UV-VIS spectrophotometer. Glass cuvettes (1 cm × 1 cm × 4.5 cm) were used for visible absorbance measurements.
(b) High performance liquid chromatographic analysis was carried out using HP 1100 HPLC system from Agilent equipped with: degasser G 1322 A, quaternary gradient pump G 1322 A, auto-sampler and UV detector G 1314 VWC at 280 nm.

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Chromatographic separation was carried out at 30 °C. On Zorbax SB-C18 column (250 mm × 4.6 mm, particle size 5 μm).

(c) Gas chromatography–mass spectrometry analysis was carried out using Perkin-Elmer autosystem XL GC equipped with ZB-5 capillary column (5% phenyl–95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 μm). Carrier gas was helium with floe rate of 1 ml/min. Oven temperature was kept at 60 °C for 10 min, and programmed to 250 °C at a rate of 3 °C/min, then isothermal at 250 °C for 5 min. The injector, GC–MS interface, ion source and Turbomass detector temperature were maintained at 250, 255, 260 and 180 °C, respectively. Mass spectra were taken at 70 eV. Emission current and photomultiplier were 200 μA and 870 V. Scan duration was 0.25 sec. and mass rang was 40–450 Da.

2.7. Determination of total phenolic contents

Total phenolic content were determined using Folin–Ciocalteu reagent, modification method by Wolfe et al. (2003). Gallic acid was used as standard and the concentration of total phenolic compounds in the extracts were calculated by standard curve interpolation. Results were reported as mg gallic acid equivalent/g dried sample. Analyses were done in triplicate.

2.8. High performance liquid chromatographic identification and quantification of phenolic compound in plant extracts

Five grams of dried plant materials were extracted by methanol (HPLC grade). The extracts of each plant were evaporated under vacuum and at room temperature to dryness. The dry methanol extracts of plants (thyme, sage and marjoram) were disolved in methanol and chromatographed under gradient conditions, with a flow rate of 0.8 ml/min. The gradient starting with 95% H_2O containing 0.05% formic acid (v/v) and 5% methanol, was kept constant for 10 min, and then the methanol concentration was changed according to the following order: 15, 30, 40, 45, 60, 80% and then decreased by 5% after 15, 20, 30, 50, 52, 60 and 65 min, respectively. The injection volume was 50 μl and chromatogram was acquired at 280 nm.

Solutions of available pure known compounds were chromatographed as external standards. All standards were dissolved in methanol before injection in the analytical HPLC system. Their ranges of concentration used were 0.7–15.0 mg/l. Phenolic compounds of plant extracts were identified by comparing their retention times with those of pure standards. The results were expressed as % of each compound from the total phenolic compounds.

2.9. Antioxidant capacity by DPPH• assay

Free radical scavenging activity were measured by using the 2,2-diphenyl-1-picrylhydrazil (DPPH•) according to a modified method by Wu et al. (2006). Sample extracts (100 μl) were added into 3.9 ml of DPPH reagent (prepared with 24 mg of DPPH/L of methanol). The percentage of DPPH scavenging activity is expressed by the following formula,

$$\text{DPPH inhibition \%} = \frac{\text{Initial absorbance} - \text{sample absorbance}}{\text{Initial absorbance}} \times 100$$

2.10. Determination of antibacterial activity

2.10.1. Disc diffusion assay

The disc diffusion method described by Lennette et al. (1980) was used for determination of antimicrobial activity of plant extracts and their essential oils, as follows: sterile nutrient agar medium (Merck) was prepared and distributed into Petri plates of 90 mm diameter. The disc diameter used was 6 mm (Whatman No. 1) paper. Different dilutions of the extracts and essential oils were made with methanol. The microbial suspension was streaked over the surface of the nutrient agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. Under aseptic conditions, the discs were placed on the agar plates and then 7.5, 10, 12.5, 15 and 20 μg from each of the extracts and essential oil dilutions was put on the discs. A dilution solvent (methanol) was added to the discs on the control plates. The plates were then incubated at optimum temperature (37 °C for bacteria and 25 °C for yeast and fungi) for 24–48 h in order to get reliable microbial growth. Diameters of microbial inhibition zones (mm) were measured and recorded.

2.10.2. Minimum inhibitory concentration (MIC) of plant extracts and essential oils

A microdilution broth susceptibility assay was used as recommended by Natural Committee for Clinical Laboratory Standard-NCCLS (1999) for the determination of the MIC. All tests were performed in nutrient broth for bacteria and in potato dextrose broth for yeast and fungi. Concentrations of 7.5, 10, 12.5, 15 and 20 μg dry extract or essential oil were added to 1 ml nutrient broth tubes containing 10^5 CFU/ml of live microorganism's cells. The tubes which contained 10 ml broth were incubated on an incubator shaker as to evenly disperse the extracts and essential oils throughout the broth in the tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC. Cells from the tubes showing no growth were subcultured on nutrient agar plates to determine if the inhibition was reversible or permanent.

2.11. Statistical analyses

Main effects means indicating significant differences were tested using Duncan's multiple range test (Duncan, 1955). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS) (2003).

3. Results and discussion

3.1. Essential oil, extract yields and total phenol content

The yield of fennel seeds and chamomile flowers essential oil and various extracts extracted by four solvents (methanol, ethanol, diethyl ether, and hexane) are given in Table 1. The yield of hydrodistilled chamomile flowers and fennel seeds essential oil were found to be 0.73% and 1.95%, respectively. Mimica-Dukic et al. (2003a,b) determined the yield of the essential oils obtained from fennel seeds by steam distillation, range 1.82–3.38%. Maximum extract yield was obtained with methanol 19.6% and minimum with hexane 5.6%. Based on these results, the extracting ability of different solvents followed the order: methanol > ethanol > diethyl ether and finally hexane. The present results demonstrated a significant ($P < 0.05$) difference in the extract yields among the solvent systems used. Our results regarding higher extract yield with methanol are in good agreement with the finding of Oktay et al. (2003) who reported 10.95% yield of fennel seed extracts with ethanol. Conforti et al. (2006) reported 10.95% and 15.78% extract yields, respectively, from cultivated and wild fennel seeds using methanol. Mata et al. (2007) found the yield of essential oil and ethanol extract of fennel seed from Portugal to be 0.1% and 6.9%, respectively.

The total phenolic contents (TPC) of chamomile flowers and fennel seed extract Table 1. The amounts of TPC extracted from chamomile flowers and fennel seeds in different solvent systems were in the ranges 3.7–2.4 GAE (mg/g) and 3.4–2.6 GAE (mg/g), respectively. Results revealed that methanol and ethanol

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Table 1
Total phenolic contents of the yield extracted aromatic plants and free radical scavenging activities expressed as EC_{50} and antiradical power (ARP), as affected by solvent extraction.

Plants	Solvents	Extract yield* (%)	Total phenols**	EC_{50}***	ARP***
Chamomile	Methanol	19.6 ± 2.6	3.7 ± 2.0	0.0022 ± 0.0005^{g}	455 ± 35.7$^{e\ f}$
Fennel		16.6 ± 2.0	3.4 ± 1.7	0.0031 ± 0.0008^{cd}	323 ± 30.$^{g\ h\ i}$
Chamomile	Ethanol	12.6 ± 2.2	3.5 ± 1.7	0.0026 ± 0.0007^{e}	385 ± 28.6$^{f\ g}$
Fennel		10.8 ± 1.1	3.0 ± 2.6	0.0036 ± 0.0011^{a}	278 ± 54.4^{k}
Chamomile	Diethyl ether	6.2 ± 2.6	3.3 ± 1.0	0.0039 ± 0.0001^{j}	256 ± 2.6^{c}
Fennel		9.4 ± 3.6	2.8 ± 1.7	0.0046 ± 0.0001^{k}	217 ± 5.8$^{l\ j\ k}$
Chamomile	Hexane	5.6 ± 4.3	2.4 ± 2.6	0.0041 ± 0.0001^{d}	244 ± 3.9$^{g\ h}$
Fennel		7.8 ± 2.6	2.6 ± 1.0	0.0047 ± 0.0001^{b}	213 ± 1.0$^{j\ k}$

* Data expressed as grams of dry extract per 100 g of dried plant material.
** Total phenols are expressed as gallic acid equivalents; mgs of gallic acid per grams of dried extract (mg GAE/g DM).
*** Means having different superscripts within the same column are significantly different at $P \leq 0.001$.

were better solvents than the others in extracting phenolic compounds from the extracts due to their polarity and good solubility for phenolic components from plant materials (Siddhuraju and Becker, 2003). Ethanol is preferred for the extraction of antioxidant compounds mainly because of its lower toxicity Karadeniz et al. (2005). Methanol extract of the chamomile and fennel showed the highest TPC, 3.7 and 3.4 GAE (mg/g), respectively. These differences in the amount of TPC may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. The ability of different solvents to extract TPC was of the order: methanol > ethanol > diethyl ether and finally hexane. Conforti et al. (2006) reported TPC (chlorogenic acid equivalents) from extracts of cultivated and wild fennel to be 100 and 151 mg/g extract, respectively. According to Mata et al. (2007) the ethanol extract of fennel seed revealed 63.1 mg/g TPC (pyrogallol equivalents).

So, it could be concluded from the results shown in the same Table 1 that the higher polar solvents were more efficient in extracting phenolic compounds from all plant materials than the less polar solvents. These results are almost similar to those reported by Hernandez-Hernandez et al. (2009). Since phenolic content estimated by the Folin–Ciocalteu procedure does not give a full picture of the quality and quantity of the phenolic constituents, HPLC analyses for determination of individual phenolic constituents is necessary.

3.2. High performance liquid chromatographic (HPLC) separation and determination of main phenolic compounds in methanolic plant extracts

Species are known to contain a range of secondary metabolites, such as terpenoids and flavonoids. Therefore qualitative quantitative analysis of the investigated methanolic plant extracts, made using high performance liquid chromatographic (HPLC) as described in the experimental part, is presented in Table 2. Representative chromatograms are shown in Fig. 1. The components of caffeic acid, quinic acid, p-coumaric acid, caffeoylquinic acid derivative, quercetin-7-o-glucoside, ferulic acid, cinnamic acid, carnosic acid, rosmarenic acid, methyl rosmarenate, apigenin, nareingenin, luteolin-7-o-rutinose and ferulic acid derivative were identified in chamomile and fennel methanolic extract by comparisons to the retention time and UV spectra of authentic standards while the quantitive data were calculated from the calibration curves. Caffeic acid was the dominant phenolic compound in chamomile methanolic extract, since it constituted 9% of the total extracted compounds. On the other hand flavonoids and flavonoid derivatives have been notice as the main components of the chamomile flowers. Apigenin-7-*o*-glycoside, quercetin, apigenin, quercetin-7-*o*-glycoside, luteolin-7-*o*-rutinose and hesperidin have been found in appreciable amounts (55% of the methanolic extract of chamomile flowers) which were more than the 45% of phenolic compound. The finding reported herin both confirm the precence

Table 2
Major phenolic compounds (% of total) identified in chamomile and fennel methanolic extract by HPLC.

Compounds	Approximate Rt (min)	% of the total	
		Chamomile	Fennel
Neochlorogenic acid	3.556	1.24	1.40
Chlorogenic acid	4.237	2.74	2.98
Gallic acid	7.543	–	0.169
Chlorogenic acid	18.772	3.240	6.873
Caffeic acid	19.687	8.970	2.960
p-Coumaric acid	22.740	3.953	4.325
Ferulic acid-7-*o* glucoside	25.578	1.493	5.223
Quercetin-7-*o* glucoside	27.031	4.354	3.219
Ferulic acid	29.580	9.201	3.555
1,5 Dicaffeoylquinic acid	32.702	–	4.095
Hesperidin	36.172	2.185	0.203
Cinnamic acid	38.443	–	0.131
Rosmarenic acid	44.999	–	14.998
Quercetin	52.981	11.563	17.097
Apigenin	56.532	6.647	12.558

of several flavonoids described by Mulinacci et al. (2000), and evidence appreciable amounts of ferulic acid, caffeic acid and caffeoylquinic acid derivative (Weber et al., 2007).

The composition of the individual phenolic compounds of the fennel seeds methanolic extract which was illustrated in Table 2 and Fig. 1 exhibited that rosmarenic acid, chlorogenic acids were the major phenolic compounds (14.9% and 6.8%, respectively). Mean while quercitin and apigenin were the most abundant flavonoid in the fennel methanol extract (17.1% and 12.5%, respectively). In spite of that the total phenolic compounds in fennel methanol extract were higher than the flavonoid compounds.

In all plant extracts, a number of components could not be identified, however their chemical class was determined from their chromatographic behavior and UV spectra. These results are in agreement with most of findings of Krizman et al. (2007) and Faudale et al. (2008). Rosmarenic acid estimated in our fennel extract was higher than those reported by the previous authers, which could be explained by the differences in growing conditions in different region and also the differences in fennel plant genotypes.

3.3. Chemical composition of the essential oil by GC–MS

The results of the chemical composition of chamomile and fennel essential oil are presented in Table 3. Overall, 15 compounds representing 95% of the oil were identified with the aid of GC–MS. The major constituents of the essential oil tested were *trans*-anethole (65.4%), fenchone (8.26%), estragole (5.2%) and limonene (4.2%). In addition, the tested fennel essential oil also contained considerable amounts of various minor constituents

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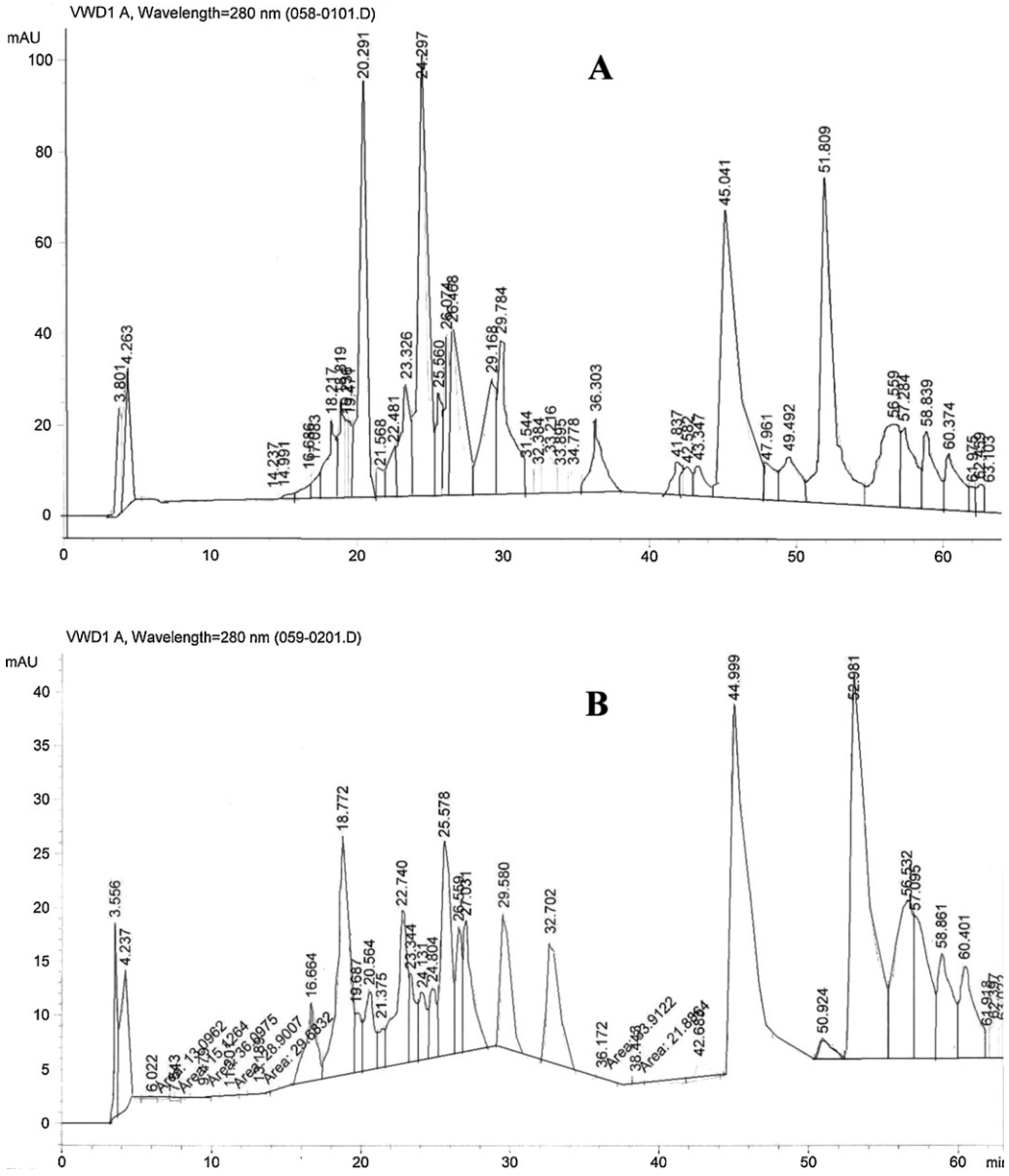

Fig. 1. HPLC chromatogram of chamomile (A) and fennel (B) methanolic extract.

whose contribution was <10%. Regarding the groups of chemical constituents represented, *Trans*-anethole, fenchone and estragole were the main oxygenated monoterpenes, while limonene was the major monoterpene. A literature search revealed *trans*-anethole (62.0%), fenchone (20.3%), estragole (4.90%) and limonene (3.15%) to be the main components of essential oils from wild-growing fennel seed native to the Podgorica region, central south Montenegro Damjanovic et al. (2005). Mimica-Dukic et al. (2003a,b) also reported *trans*-anethole (74.18%), fenchone (11.32%), estragole (5.29%), limonene (2.53%) and α-pinene (2.77%) as the major compounds identified in the essential oil from *F. vulgare* Mill. Ozcan et al. (2006) reported estragole (61.08% and 40.49%), fenchone (23.46% and 16.90%) and limonene (8.68% and 17.66%), respectively, as the major constituents in the essential oil of bitter fennel (*F. vulgare* spp. *piperitum*) grown in Turkey. Such variations in the chemical composition of essential oil across countries might be attributed to the varied agroclimatic (climatical, seasonal, geographical) conditions of the regions, stage of maturity and adaptive metabolism of plants.

The steam distillation of chamomile flowers yielded oil with a distinct smell and light green in color. The constituents of the essential oil were analyzed by GC–MS. Total 13 compounds representing 97.5% of the oil were identified and the results are listed in Table 3. The major constituents detected were: α-bisabolol oxide A (48.22%), α-bisabolol oxide B (23.31%), α-bisabolol (12.1%) and β-farnesene (5.21%).

3.4. Antioxidant activities using DPPH• radical scavenging

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman et al., 2003). The DPPH• is considered to be a model of a stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. Antioxidants react with DPPH•, reducing a number of DPPH• molecules equal to the number of their available hydroxyl groups. Therefore, the absorption at 515 nm was

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Table 3
Relative percentage composition of chamomile and fennel essential oil by GC–MS analysis.

Compounds	% of total	
	Chamomile	Fennel
α-Pinene	0.71	1.6
β-Myrcen	0.25	0.62
α-Terpinin	0.5	0.58
Limonene	0.8	4.2
γ-Terpinen	0.1	1.4
Methyl chavicol	–	5.2
1.8-Cineol	0.2	0.89
Fenchone	–	8.26
Estragole	–	5.21
Trans-Anethol		56.4
Terpin-4-ol	1.7	2.8
Myrcenol	–	1.02
Bergamoil	–	0.63
2,5-Diethyl phenol	–	0.78
β-Farnesene	5.21	3.01
α-Farnesene	–	1.28
P-cymene	2.01	–
α-Bisabolol oxide A	48.22	–
α-Bisabolol oxide B	23.31	–
α-Bisabolol	12.1	–
Chamazulene	2.42	–

proportional to the amount of residual DPPH• (Juan et al., 2005). It is visually noticeable as a discoloration from purple to yellow.

The amount of plant extract needed to decrease the initial DPPH• concentration by 50% (EC_{50}) is a parameter widely used to measure the antioxidant activity. Usually (EC_{50}) is defined as: [the moles of phenolic compounds divided by moles of DPPH• necessary to decrease by 50% the absorbance of DPPH•]. The lower the EC_{50}, the higher is the antioxidant power. Another parameter is the anti-radical power (ARP) which is calculated by dividing 1 by EC_{50}. The higher the value of antiradical power (ARP), the higher is the free radical scavenging activity. Free radical scavenging capacity increased with increasing extract concentration Table 1.

Plant extracts showed excellent radical scavenging activity, with IC_{50} values ranging from 0.0022 to 0.0041 and from 0.0031 to 0.0047 for chamomile and fennel, respectively (Fig. 2). The free radical scavenging activity of methanol and ethanol extracts were superior to that of other solvents. Furthermore, methanol extracts exhibited more scavenging activity than ethanol extracts.

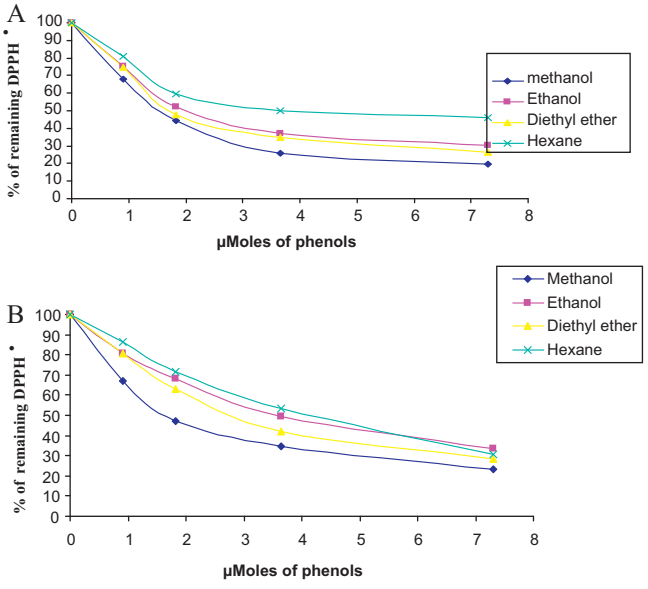

Fig. 2. Scavenging activities of chamomile (A) and fennel (B) phenolic compounds as influenced by extracting solvents.

Statistically, the interaction between plant materials and solvents significantly affected ($P \leq 0.001$) both EC_{50} and consequently ARP values. There were great differences among the free radical scavenging activities of different extracts of plant materials. Regarding plant effect chamomile had the highest ARP of 455 followed by fennel 385. Concerning solvents, regardless of plants, methanol had the highest ARP followed by diethyl, ethanol and hexane in a descending order being 455, 385, 256 and 244 for chamomile and 323, 278, 217 and 213 for fennel, respectively.

Free radical scavenging activity of chamomile methanol extracts could be due to their higher content of phenolic components which were chlorogenic acid, Caffeic acid, p-coumaric acid and ferulic acid according to the HPLC analysis (Table 2 and Fig. 1). Such hydroxyl-phenolic compounds can donate hydrogen atoms to DPPH• and scavenge it. Such explanation was introduced by Lu and Foo (2001).

3.5. Antimicrobial activity of essential oils and plant extracts

3.5.1. Estimation of antimicrobial activity of essential oils and plant extracts by disc diffusion method

Food poisoning originating from contaminated foods by both Gram-positive and Gram-negative bacteria causes concern to society and to industry. A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment (Valero and Salmeroj, 2003). Spices and herbs have been safely since ancient times as food flavoring agents and also as herbal medicines and are now mainly considered "generally regarded as safe" (GRAS). Recently there have been considerable emphasis studies involving essential oils and extracts of spices and herbs on inhibiting the growth of microbes (Falzari and Menary, 2003).

Besides, Gulfraz et al. (2008) reported that the essential oil and seed extracts of *F. vulgare* could be a source of pharmaceutical materials required for the preparations of new therapeutic and antimicrobial agents. Therefore, this piece of work has been undertaken and used the disc diffusion technique to estimate the antimicrobial activity of essential oils and different extracts of chamomile and fennel. The antimicrobial activity of the essential oils and plant extracts were examined in the disc diffusion assay against a panel of six microorganisms selected on the baise of their relevance to public health. The data expressed as diameter of growth inhibition zone (mm). The results are illustrated in Tables 4 and 5.

Variable zones of microbial growth inhibition were noted in both of the plant essential oils (Fig. 3). Zones of growth depend on the concentration of essential oil. The essential oil from chamomile were strongly bactericidal than the essential oil from fennel. The concentration of 20 μg/disc was the best concentration as antimicrobial activity. Chamomile and fennel extracts exhibited considerable antimicrobial activity against all the strains tested, particularly against Gram-positive bacteria. Methanol extract was the higher effect as antimicrobial activity than other extracts (Table 4).

3.5.2. Determination of antimicrobial activity of essential oils and plant extracts by agar dilution method (MIC)

The minimal inhibitory concentration (MIC) of chamomile and fennel essential oils and their extracts were determined in order to assess their antimicrobial activity. Essential oil of chamomile showed lower MIC values (12.5–10), compared to fennel (15–12.5), for all strains tested, indicating their antimicrobial activity (Table 5). The minimal inhibitory concentration (MIC) of chamomile and fennel extracts depended on the bacterium being studied and the extract concentration. The MIC values

Table 4
Antimicrobial activity of plants extracts using disc diffusion assay (inhibition zone diameter in mm[a]).

Plants	Conc. μg/disk	Microorganisms					
		E. coli O157	*S. tyhpi*	*B. cereus*	*S. aureus*	*A. flavus*	*C. albicans*
Methanolic extracts							
Chamomile	7.5	8	11	9	11	18	15
	10	9	12	13	15	17	17
	12.5	12	14	16	15	21	19
	15	15	18	18	16	22	21
	20	18	20	20	19	24	23
Fennel	7.5	10	10	11	10	14	13
	10	9	11	11	13	15	16
	12.5	14	15	15	16	20	18
	15	16	15	13	16	21	20
	20	17	18	18	19	22	22

Plants	Conc. μg/disk	Microorganisms					
		E. coli O157	*S. tyhpi*	*B. cereus*	*S. aureus*	*A. flavus*	*C. albicans*
Ethanolic extracts							
Chamomile	7.5	9	8	10	13	11	8
	10	10	11	13	15	13	11
	12.5	12	10	12	13	13	13
	15	16	14	18	20	15	19
	20	19	17	20	23	18	21
Fennel	7.5	8	7	7	6	7	8
	10	9	10	10	12	12	10
	12.5	10	9	12	13	13	12
	15	10	12	13	13	15	18
	20	15	15	17	19	18	17

Plants	Conc. μg/disk	Microorganisms					
		E. coli O157	*S. tyhpi*	*B. cereus*	*S. aureus*	*A. flavus*	*C. albicans*
Diethyl ether extract							
Chamomile	7.5	7	6	7	8	6	8
	10	8	8	9	10	9	10
	12.5	11	11	12	14	13	14
	15	15	12	14	17	16	16
	20	15	16	18	19	21	20
Fennel	7.5	7	8	8	8	9	9
	10	8	7	10	10	8	10
	12.5	11	12	12	13	12	14
	15	13	14	14	15	16	15
	20	15	15	17	17	19	19

Plants	Conc. μg/disk	Microorganisms					
		E. coli O157	*S. tyhpi*	*B. cereus*	*S. aureus*	*A. flavus*	*C. albicans*
Hexane extracts							
Chamomile	7.5	8	8	9	10	8	9
	10	9	10	15	15	12	15
	12.5	15	13	17	18	15	19
	15	17	16	19	21	18	22
	20	19	19	21	23	20	23
Fennel	7.5	7	8	9	9	8	8
	10	8	10	11	15	10	11
	12.5	13	12	13	18	12	13
	15	16	14	15	21	16	16
	20	19	17	18	23	19	20

[a] The diameter (mm) of the inhibition zone is the mean of three independent experiments including the diameter of the paper disc (4 mm).

of chamomile, generally ranged from (10–17.5), with the exception being *E. coli*, *S. typhi* and *A. flavus* which required higher concentration.

4. Discussion

The antioxidant activities of the different extracts were evaluated by measuring the scavenging activity of these extracts toward the stable 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH•) free radicals. Among the extracts examined, chamomile methanolic extract exerted the best antioxidative activities. The statistical analysis showed that the highest antiradical power (ARP) was noticed for Chamomile extracted by methanol regardless of treatment, being 455.

The growth of food spoilage and food-borne pathogens on/in food can decrease nutritional quality of the food by consuming fat, protein and carbohydrate that are present in the food, subsequently causes food discoloration, heating, mustiness, biochemical changes, weight loss and toxicity. Some species of them are able to produce highly toxic compounds which can adversely affect the health of humans (Li and Yi, 2003; You, 2006).

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Table 5
Minimum inhibitory concentration (MIC, μg ml^{-1}) of plants essential oil and extracts against some pathogenic microorganisms.

Plants	Extracts	Microorganisms					
		E. coli	*S. typhi*	*B. cereus*	*S. aureus*	*A. flavus*	*C. albicans*
Chamomile	Methanol	15	15	12.5	12.5	12.5	10
	Ethanol	15	15	12.5	12.5	15	12.5
	Diethyl ether	17.5	15	15	15	17.5	15
	Hexane	15	15	12.5	10	12.5	10
	Essential oil	12.5	12.5	10	10	12.5	10
Fennel	Methanol	15	15	12.5	12.5	10	10
	Ethanol	15	15	15	15	12.5	10
	Diethyl ether	15	17.5	15	15	12.5	15
	Hexane	12.5	15	12.5	10	10	12.5
	Essential oil	12.5	15	12.5	10	10	10

Means have different superscripts within each effect within the same column are significantly different at $P \leq 0.001$.

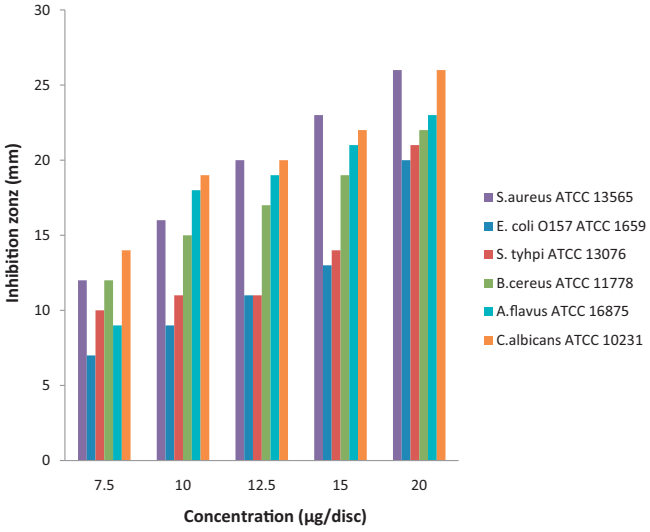

Fig. 3. Antimicrobial activity of plant essential oils, using disc diffusion assay (inhibition zone diameter in mm*). *The diameter (mm) of the inhibition zone is the mean of three independent experiments including the diameter of the paper disc (4 mm).

The method of microbial growth inhibition most appropriate to food is the use of food preservatives. An ideal food preservative must be inexpensive, corrosion-free, low in toxicity, and have good antimicrobial activity. The inhibitors available for practical use today are mainly chemical preservations. However, the safety problems with chemical preservatives are receiving growing attention, and natural preservatives for foods have high potential for the food industry (Li and Yi, 2003; Xie et al., 2001).

Many extracts from medicine plant have been known to possess antimicrobial effects and used for the purpose of food preservation and medicinal purposes (Cowan, 1999; Lee et al., 2007; Tassou et al., 2000; Valero and Salmeroj, 2003). In this study, the extracts obtained by different solvents displays effective antimicrobial effects against food spoilage and food-borne pathogens and broad antimicrobial spectrum.

It has been reported in some literatures that such materials as flavonoids (*e.g.* quercetin, apigenin and hesperidin), and volatile oil can be obtained from chamomile and fennel. These active ingredients produce varied pharmacological effects such as disinfection, ant inflammation, anti-neoplasm, immunoregulation, liver and damage protection, blood glucose decrease (Yan et al., 2003). The application of the extracts of chamomile and fennel in food industry not only facilitates antioxidant and antimicrobial activities, but also contributes to such pharmacological activities as food preservation, healthcare as well as food nutrients. Therefore, it is a natural food additive with considerable market prospect.

The results from the disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that *B. cereus* and *A. flavus* were the most sensitive microorganisms tested, showing the largest inhibition zones and the lowest MIC values. Least activity was exhibited against *E. coli*, with the smallest inhibition zones and the highest MIC value. In agreement with our results, Cantore et al. (2004) reported that the Gram-negative strains of bacteria, especially *E. coli*, have less sensitivity to chamomile and fennel essential oils. Ozcan et al. (2006) found that fennel essential oils exhibit an inhibitory effect against a wide range of *Bacillius* species. Mimica-Dukic et al. (2003a,b) also reported that the essential oils of fennel are active against *Aspergillus* species. Ozcan et al. (2006) reported the antifungal activity of essential oils from bitter fennel. As expected, fennel seed extracts offered antimicrobial activity.

The results of the present study demonstrated that essential oil and various extracts from chamomile and fennel show good antioxidant and free radical scavenging activities. Furthermore, essential oil also exhibited appreciable antimicrobial activity. The production of such essential oil and bioactive components from indigenous resources and their utilization as potential natural food preservatives could be of economic value. However, further investigations involving more detailed *in vitro* and *in vivo* studies to establish which components of the essential oil or extracts offer the best antioxidant activity are recommended. Overall, this study presents valuable information on the composition and antioxidant attributes of chamomile and fennel essential oil and their extracts from Egypt. It advocates its consumption in food and pharmaceutical preparations local industries. In addition, fennel showing antioxidant activity might be explored for functional food and nutraceutical applications, besides its traditional uses.

5. Conclusion

In these results we have focused on extracts obtained from chamomile and fennel commonly used for their antioxidant properties and antimicrobial activity, which have rarely been reviewed. Results of these antioxidant activities antimicrobial activity of selected spices using DPPH assay, was presented. This analysis technique could provide insight into the variations in the antioxidant profiles between different spices and could help disease prevention and cure using simple herbs and spices. Several spices were found to have high levels of antioxidant capacity and total phenolic compounds. Chamomile and fennel showed highest total phenolic content, associated with the relatively higher antioxidant activities.

The antimicrobial activity of plants essential oils and their extracts were assessed by using disc diffusion as well as minimum inhibitory concentration (MIC) method. The diameter of growth inhibition zone (mm) for chamomile methanol extract was better than fennel especially against *A. flavus*, *C. albicans* and

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Gram-positive bacteria. The lowest MIC values of essential oils for *A. flavus*, *C. albicans*, *B. cereus*, and *S. aureus* was obtained. The essential oils exhibit different degrees of antimicrobial activities depending on the doses applied. Therefore, essential oil and extracts of the investigated plant materials could be recommended as source of pharmaceutical materials required for the preparation of new antimicrobial agents.

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