Arch Lebensmittelhyg 67,
17–24 (2016)
DOI 10.2376/0003-925X-67-17

ISSN 0003-925X

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Investigation of antimicrobial and antioxidant activities of essential oils extracted from medicinal plants

Untersuchung der antimikrobiellen und antioxidativen Aktivitäten von ätherischen Ölen extrahiert aus verschiedenen Heilpflanzen

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Summary

Essential oils of eleven plants including anise, hyssop, flos lavandulae, pot marigold, fennel, mint, chamomile petal, clary sage, cilantro, herba lippiae and dill were screened for total phenolic content by the Folin-Ciocalteu method, for potential antioxidant activity using the phosphomolybdenum assay and for antiradical activity by the 1,1-diphenyl-2-picryl hydrazyl (DPHH) method. The antimicrobial activity was examined by using agar disc diffusion as well as the minimum inhibitory concentration (MIC) method. The essential oils showed considerable antimicrobial activity against most of the tested microorganisms. The essential oil of dill was very effective in inhibiting the growth of all bacterial strains tested, with a low MIC (125 µl/ml). Overall, the lowest MIC was found for *E. coli* O157:H7 and *E. coli* ATCC 25922 while the highest values were found for *S. aureus* and *L. monocytogenes.* The highest total antioxidant capacity as ascorbic acid equivalent (AAE) of 195.27 mg/g was obtained for mint with the phosphomolybdenium assay. The highest percentage of inhibition of DPPH radical was obtained with essential oil from dill (92.70 %). It was followed by radical scavenging activities of essential oils from mint (81.00 %) and anise (71.53 %). Total phenolic content of the essential oils ranged from 2.33 to 695.06 mg gallic acid per 100 g of the samples. Mint and dill had the highest content of total phenols. The essential oils of mint, dill, anise hyssop, and flos lavandulae may prove to be a good source of antioxidant and antimicrobial agents for the food and pharmaceutical industries.

Keywords: Antibacterial activity, antioxidant, total phenolic

Zusammenfassung

In dieser Studie wurde der Gesamtphenolgehalt mit Hilfe der Folin-Ciocalteu-Methode, die potenzielle antioxidative Aktivität mittels Phosphormolybdän-Methode sowie die Antiradikalaktivität unter Nutzung der 1,1-Diphenyl-2-picryl hydrazyl (DPPH)-Methode in ätherischen Ölen aus elf Pflanzen (Anis, Ysop, Lavandelblüte, Ringelblume, Fenchel, Minze, Kamille (Blütenblätter), Salbei, Koriander, Lippienkraut und Dill) untersucht. Die antimikrobielle Aktivität wurde unter Verwendung des Agardiffusionstests sowie der minimalen Hemmstoffkonzentration (MHK) untersucht. Die ätherischen Öle zeigten eine beträchtliche antimikrobielle Aktivität gegenüber den meisten getesteten Mikroorganismen. Das ätherische Öl von Dill hemmte das Wachstum aller untersuchten Bakterien sehr wirksam (MHK 125 µl/ml). Der niedrigste MHK wurde für *E. coli* O157:H7 und *E. coli* ATCC 25922 ermittelt. Die höchsten Werte wurden für *S. aureus* und *L. monocytogenes* festgestellt. Die höchste antioxidative Aktivität wurde für Minze im Phosphormolybdän-Test ermittelt (Ascorbinsäure-Äquivalent (AAE) von 195,27 mg/g). Der größte prozentuale Anteil (%) der Hemmung der DPPH-Radikalen wurde aus dem ätherischen Öl von Dill (92,70 %) erzielt. Gefolgt von den ätherischen Ölen der Minze (81,00 %) und Anis (71,53 %). Der Gesamtphenolgehalt der ätherischen Öle reichte von 2,33 bis 695,06 mg Gallussäure pro 100 g Probe. Minze und Dill hatten den höchsten Gehalt an Phenolen. Die Ergebnisse zeigen, dass die ätherischen Öle von Minze, Dill, Anis, Ysop und Lavendelblüten eine gute Quelle für antioxidativ und antimikrobiell wirkende Mittel für die Lebensmittel- und Pharmaindustrie darstellen können.

Schlüsselwörter: Antibakterielle Aktivität, Antioxidantien, Gesamtphenolgehalt

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Introduction

Preservation of food materials from degradation, mainly by the activity of microorganisms during production, storage and marketing is an important issue in the food industry (Viuda-Martos et al., 2011). The control of food spoilage and pathogenic microorganisms is achieved mainly by chemical control but the use of synthetic chemicals is limited due to a number of undesirable aspects. Recently, because of the resistance that pathogens build against antibiotics and the demand from consumers for 'green' products that do not include any synthetic chemicals, natural antimicrobials such as herbs and spices and especially their derivatives such as essential oils are increasingly being considered for food preservation (Xu et al., 2007). 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).

During the storage of raw materials, processing, heat treatment and further storage of the final products, oxidation is another deterioration process. Oxidation may cause rancidity in food products, leading to the degradation of lipids and proteins; thereby contributing to the loss in flavour, texture and colour of food products. The odours and flavours resulting from oxidation can easily destroy the organoleptic and nutritional quality of processed foods (Karpinska et al., 2001).

Phenolic compounds exhibit considerable antioxidant activities due to the potential loss of a hydrogen atom and/or a single electron and metal chelating properties (Rice-Evans et al., 1996, 1997). These compounds are frequently found as secondary metabolites in various plant species, including edible and medicinal ones and the relation between the therapeutic potential of the plant species and their phenolic content has been emphasised in many publications (Dai and Mumper, 2010; Santos-Buelga et al., 2012; Çekiç et al., 2013; Walch et al., 2011). Therefore, qualitative and quantitative determination of these compounds in plants extracts and evaluation of their biological activities are undoubtedly very important.

Medicinal plants used in traditional medicines seem to be a rich source of natural and safe biocidal metabolites. Essential oils (also called volatile oils) are natural volatile complex compounds that are characterized by a strong smell and are formed as secondary metabolites in edible, medicinal and herbal plants (Burt, 2004). Their components represent a source of natural antioxidants and antimicrobial substances and have the potential to be used in the food industry to increase the shelf life of food products without any side effects (Tajkarimi et al., 2010; Solorzano-Santos and Miranda-Novales, 2012). A variety of studies have been carried out to evaluate the antimicrobial and antioxidant activities of essential oils and the results indicate that essential oils do have noticeable antimicrobial and antioxidant activities (Bakkali et al., 2008; Rahman and Kang, 2009). In the last decades, essential oils and various extracts of plants (e.g. fennel, sage, thymus, grape etc.) have been of great interest as sources of natural antioxidants.

In this study, we have aimed to investigate the antibacterial activity, antioxidant properties and phenolic compounds of essential oils extracted from a wide range of plants including anise, hyssop, flos lavandulae, pot marigold, fennel, mint, chamomile petal, clary sage, cilantro, herba lippiae and dill. The plants used in this study were selected on the basis of traditional and widespread culinary or domestic use.

Material and Methods

Material

Plant materials

The plant samples were grown in the University of Selçuk, Çumra Vocational School, Department of Medical and Aromatic Plants, Konya, Turkey and collected between spring and summer of 2014. The plant materials were air dried at room temperature under the shade for 10 days. The identities and parts of the plants used to obtain essential oils are given in Table 1.

Methods

Isolation of essential oils

The essential oils were obtained by hydrodistillation using a Clevenger type apparatus according to Olmedo et al. (2012). Dried plant materials (100 g) were cut into small pieces and placed in a distillation apparatus with 2 : 1 of double distilled water and hydro-distilled for 3 h. After the oils were dried over anhydrous sodium sulphate, they were stored at 4 °C until analysis.

Antimicrobial activity

Microbial strains

The essential oils were individually tested against five pathogenic microorgansms: *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* subsp. *enterica serovar* Enteritidis ATCC 13076, and *Staphylococcus aureus* ATCC 2592. All the strains mentioned above were obtained as actively growing cultures from the American Type Culture Collection (ATCC). Stock cultures of all the strains were grown in Nutrient Broth (Acumedia Manufactuers, Inc., Maryland) at 37 °C for 24 h and suspensions were adjusted to 0.5 McFarland standard turbidity (each bacterial suspension included about 10^7–10^8 cfu per mlcells).

TABLE 1: *The identity of plant species from which essential oils were extracted.*

Plant Name	Botanical Name	Family	Part Used
Anise	*Pimpinella anisum*	Apiaceae	Fruits
Hyssop	*Hyssopus officinalis* L	Lamiaceae	Flower-Pedicel-Leaves
Flos Lavandulae	*Lavandula angustifolia* Mill.	Lamiaceae	Flower-Pedicel-Leaves
Pot Marigold	*Calendula officinalis* L.	Asteraceae	Flower
Fennel	*Foeniculum vulgare* Mill.	Apiaceae	Fruits
Mint	*Mentha spicata*	Lamiaceae	Leaves
Chamomile petal	*Anthemis nobilis* L. (Syn: Chamaemelum nobile All.)	Asteraceae	Flower
Clary sage	*Salvia sclarea* L.	Lamiaceae	Flower-Leaves
Cilantro	*Coriandrum sativum* L.	Apiaceae	Flower-Pedicel
Herba lippiae	*Melissa officinalis* L.	Lamiaceae	Leaves
Dill	*Anethum graveolens* L.	Apiaceae	Seed

Disc diffusion assay

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oils (NCCLS, 1997). Briefly, a suspension of the tested microorganism (0.1 ml of 10^8 cfu per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µl of the oil or dimethyl sulfoxide (DMSO; negative control) and placed on the inoculated plates. These plates were stored at 4 °C for 2 h and then incubated at 37 °C for 24 h for the observation of bacterial growth. The diameters of the inhibition zones were measured in millimeters. All tests were performed in duplicate. The antibiotic gentamicin (10 µg/disc) was used as a positive control.

Determination of minimum inhibitory concentration (MIC)

Bacterial strains sensitive to the plant oils in disc diffusion assay were studied for their minimal inhibition concentration (MIC) values using the micro-well dilution assay method (Gulluce et al., 2004). MIC is defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganism. For the determination of antibacterial activities, *E. coli* ATCC 25922, *E. coli* O157:H7, *L. monocytogenes* ATCC 7644, *S. Enteritis* ATCC 13076, and *S. aureus* ATCC 25923 were used as target bacteria.

The inoculated microbial strains were prepared from 12 h broth cultures and the suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils, dissolved in 10 % (v/v) DMSO, were first diluted to the highest concentration (1000 µg/ml) for testing, and then serial two-fold dilutions were made between the concentrations of 62.5 and 1000 µg/ml in 10 ml sterile test tubes containing Mueller-Hinton Broth (MHB) (Merck-Darmstadt, Germany).

The culture medium (95 µl) and 5 µl of the bacterial inoculum were dispensed to each well of a 96-well plate. A 100 µl aliquot from the stock solutions of the essential oils initially prepared at the concentration of 1000 µg/ml was added into the first wells. Then, 100 µl of their serial dilutions were transferred into seven consecutive wells. The last well, containing 195 µl of nutrient broth without any essential oil and 5 µl of the inoculum on watch strip, was used as the negative control. The final volume in each well was 200 µl. The contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated for 24 h at 37 °C.

Microbial growth was determined by the presence of a white pellet in the bottom of the well and confirmed by plating 5 µl samples from clear wells on Nutrient Agar (NA) medium (Acumedia Manufacturers, Inc., Maryland). The MIC value was defined as the lowest concentration of the essential oil required for inhibiting the growth of each microorganism. All tests were repeated two times.

Determination of total phenolic content

The total phenolic content was determined by using the Folin-Ciocalteu reagent and gallic acid as the standard as described previously with some modifications (Wolfe et al., 2003). Briefly, 5 ml water, 1–3 ml sample and 0.5 ml Folin-Ciocalteu Reagent were mixed and incubated for 5–8 min at room temperature. To this, 1.5 ml sodium carbonate (20 %, w/v) was added to obtain a final volume of 10 ml. The solution was mixed, incubated for 2 h and filtered (0.45 µm poly-tetrafluoroethylene filter, Whatman), prior to reading the absorbance at 750 nm in a spectrophotometer (Shimadzu UV-Vis Mini 1240). The essential oils were diluted in 50 % ethanol solution. The negative controls included 50 % ethanol without the essential oil. The total phenol content was quantified by comparing the absorbance of the samples with the absorbance of the gallic acid standard. A calibration curve with gallic acid was prepared in the 5–25 mg/l range, and results were expressed as mg of gallic acid per g of sample. All experiments were performed in triplicate.

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was used to determine the hydrogen atom- or electron-donation ability of the corresponding essential oils. This spectrophotometric assay uses the stable purple-coloured 2,20-diphenylpicrylhydrazyl (DPPH) radical as a reagent that is bleached in the presence of scavengers (Cuendet et al., 1997; Burits and Bucar, 2000). Various concentrations of the oils in methanol (50 µl final volume) were added to 5 ml of a 0.004 % (v/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of DPPH free radical as a percentage (I%) was calculated with the following equation:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound. The oil concentration providing 50 % inhibition (EC_{50}) was calculated from the graph plotted with the inhibition percentage against the oil concentration. The amount of plant essential oils needed to decrease the initial DPPH concentration by 50 % (EC_{50}) is a parameter that is 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 the absorbance of DPPH by 50 %. The lower the EC_{50}, the higher is the antioxidant power. The tests were carried out in triplicate.

Evaluation of total antioxidant capacity by the phosphomolybdenum method

The antioxidant activities of essential oil samples were evaluated by the phosphomolybdenum method and expressed relative to that of ascorbic acid (Prieto et al., 1999). A 0.4 ml aliquot of the sample in methanol was mixed with 4 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. The solvent methanol (0.4 ml) was used instead of the samples as a blank. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm. The antioxidant activity was calculated as ascorbic acid equivalents (mg AAE/g).

Results and Discussion

Antimicrobial activity of essential oils

The antimicrobial activities of 15 essential oils from eleven plants were evaluated against five pathogenic microorganisms and their inhibitory potencies were qualitatively and

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quantitatively assessed by the presence or absence of inhibition zones and MIC values. The results are given in Table 2. The antimicrobial activities of the essential oils were compared with a standard antibiotic (gentamicin), which was used as a positive control.

The results obtained from the disc diffusion method, followed by measurements of the minimum inhibitory concentration (MIC), indicated that the microorganisms *E. coli* ATCC 25922 and *E. coli* O157:H7 were the most sensitive, showing the lowest MIC values (62.25 µg/ml) in the presence of oils isolated from hyssop (leaf and flower), flos lavandulae (flower), and fennel (Tab. 2).

The essential oil most effective against bacteria was extracted from dill, which inhibited all strains of bacteria used in the study (Tab. 2). This was followed by hyssop (flower), hyssop (leaf), flos lavandulae (flower), flos lavandulae (leaf) and fennel, which inhibited four strains. The essential oil of hyssop (leaf) and flos lavandulae (flower) was ineffective against *L. monocytogenes* while that from from hyssop (flower) was ineffective against *E. coli.* Essential oils from mint and anise inhibited three strains; followed by pot marigold and flos lavandulae (pedicle), which inhibited one strain. On the other hand, oils obtained from hyssop (pedicle), clary sage, cilantro, herba lippiae and chamomile had no inhibitory effects on the bacteria.

TABLE 2: *Antimicrobial activity of the essential oils.*

Essential Oils		Test bacteria				
		E. coli ATCC 25922	*E. coli* O157:H7	*S. Enteritidis* ATCC 13076	*L. monocytogenes* ATCC 7644	*S. aureus* ATCC 2592
Anise	DD	12.0	–	11.3	–	12.5
	MIC	500	–	–	–	500
Hyssop (flower)	DD	–	23.7	17.7	11.4	15.7
	MIC	–	62.25	125	125	500
Hyssop (pedicle)	DD	–	–	–	–	–
	MIC	–	–	–	–	–
Hyssop (leaf)	DD	24.2	21.0	18.8	–	17.9
	MIC	62.25	125	250	–	500
Flos Lavandulae (flower)	DD	16.6	14.2	22.0	–	17.8
	MIC	62.25	125	125	–	250
Flos Lavandulae (pedicle)	DD	–	–	–	9.9	–
	MIC	–	–	–	250	12.4
Flos Lavandulae (leaf)	DD	12.5	14.8	–	9.7	–
	MIC	125	500	–	500	250
Pot Marigold	DD	–	–	–	–	11.1
	MIC	–	–	–	–	500
Fennel	DD	9.8	7.2	4.2	–	6.4
	MIC	–	62.25	–	–	62.25
Mint	DD	11.8	–	–	4.9	5.2
	MIC	–	–	–	125	125
Chamomile petal	DD	–	–	–	–	–
	MIC	–	–	–	–	–
Clary sage	DD	–	–	–	–	–
	MIC	–	–	–	–	–
Cilantro	DD	–	–	–	–	–
	MIC	–	–	–	–	–
Herba lippiae	DD	–	–	–	–	–
	MIC	–	–	–	–	–
Dill	DD	11.0	14.5	15.9	8.5	18.7
	MIC	125	125	125	125	125

(–): No inhibition zone and/or MIC value measured; DD: disc diffusion method in millimetres; MIC: minimum inhibitory concentrations in micro gram per millilitres.

Inhibition zones with diameters more than 20 mm were observed by adding 20 µl of the following test substance to an inoculum of 10^8 cfu/ml of microorganisms: hyssop (leaf) against *E. coli* (zone diameter 24.2 mm), hyssop (flower) against *E. coli* O157:H7 (zone diameter 23.7mm), flos lavandulae (flower) using a Enteritis strain (zone diameter 22.0 mm) and hyssop (leaf) using *E. coli* O157:H7 (zone diameter 21.0 mm).

S. aureus was the most susceptible microorganism that could be inhibited with nine of the essential oils, followed by *E. coli* ATCC 25922 and *S. Enteritis* ATCC 13076, which were inhibited by seven of the essential oils. The Gram-negative bacteria *S. Enteritis* and *E. coli* were sensitive to seven out of 15 (43.75 %) tested essential oils, while *E. coli* O157:H7 was sensitive to five (31.25 %) of the essential oils.

For *E. coli* ATCC 25922, the inhibition zones and MIC values indicated robust antimicrobial activities of essential oils from hyssop (leaf) and flos lavandulae (flower) with zone sizes of 24.2 and 16.6 mm respectively and MIC of 62.25 µg/ml. This was followed by lavandulae (leaf) and anise, which showed zones of 12.5 and 12.0 mm respectively and MIC values between 125 and 50. Antimicrobial activity against *E. coli* O157:H7 was manifested as zone diameter between 7.2–23.7 mm with MIC value of 62.25–125 depending on the essential oil sample studied. *E. coli* O157:H7 was more susceptible to essential oils from hyssop (flower) (zone diameter 23.7 mm). Most of the essential oils from plants like anise, hyssop (pedicle), flos lavandulae (pedicle), flos lavandulae (leaf), pot marigold, mint, chamomile, clary sage, cilantro and herba lippiae did not show bactericidal properties against this microorganism under the treatment conditions tested.

S. Enteritis was found to be the most susceptible to flos lavandulae (flower) essential oil with an inhibition zone of 22.0 mm. The bactericidal effectiveness against *S. Enteritis* was as follows: flos lavandulae (flower) > hyssop (leaf) > hyssop (flower) > dill > flos lavandulae (leaf) > anise> fennel.

Among the two Gram-positive bacterial strains, *S. aureus* was found to be more sensitive than *L. monocytogenes* with susceptibility seen with nine (56.25 %) and five (31.25 %) of the essential oils, respectively. The bacteriostatic activity of the three essential oils: dill, hyssop (leaf) and flos lavandulae (flower) was higher (MIC ranging from 125–500, inhibition zone 18.7, 17.9 and 17.8 mm, respec-

tively) compared to the other samples against *S. aureus.* Essential oil from medical narcissus showed inhibitory effect only on *S. aureus. L. monocytogenes* was the most resistant microorganism tested, showing the smallest inhibition zones and the highest MIC values. *L. monocytogenes* was, however, inhibited by essential oils from hyssop (flower), hyssop (leaf), flos lavandulae (pedicle), flos lavandulae (leaf), mint and dill with the greatest efficacy observed with hyssop (flower) essential oils (inhibition zone size 11.4 mm, 125 MIC). Overall, the lowest MIC was found for *E. coli* O157:H7 and *E. coli* ATCC 25922 while the highest values were found for *S. aureus* and *L. monocytogenes.*

The active antimicrobial compounds of essential oils are known to be terpenes e.g. eugenol, thymol, and carvacrol, which are phenolic in nature (Marino et al., 2001). Essential oils of dill and fennel are rich in phenolic compounds, which are believed to be responsible for the marked antimicrobial activity. Therefore, it would seem reasonable that their mode of action might be related to those of other phenolic compounds. In fact, the phenolic compounds are capable of dissolving within the bacterial membrane and thus penetrating inside the cell, where they interact with cellular metabolic mechanisms (Marino et al., 2001).

Corroborating the data presented in the current study, lavender and hyssop essential oils have previously been reported to possess antimicrobial properties (Mazzanti et al., 1998; Rota et al., 2004; Soylu et al., 2006; Romeo et al., 2009; Imelouane et al., 2009; Mahboubi and Feizabadi, 2009; Hanamanthagouda et al., 2010).

Essential oils from dill, coriander, cilantro and eucalyptus were separated into heterogeneous mixtures of components by fractional distillation and MIC values were determined against a range of microorganisms. Essential oil from dill had the lowest overall activity against the test microorganisms. However, distilled fractions containing higher concentrations of active compounds were more effective. Both Gram-negative and positive bacteria were inhibited by D-limonene. Cilantro oil strongly inhibited Gram-positive bacteria and *S. cerevisiae,* but had little effect against Gram-negative bacteria (Delaquis et al., 2002).

Marino et al. (2001) tested essential oils from mint, hyssop and chamomile for their inhibitory effects against nine strains of Gram-negative bacteria and six strains of Gram-positive bacteria. The essential oils of mint, hyssop and chamomile showed bacteriostatic activity. The bacteriostatic activity was more marked against Gram-positive bacteria while the bactericidal activity was greatest against Gram-negative bacteria. The antimicrobial activity of fennel essential oil was assessed by using the disc diffusion method (Gulfraz et al., 2008) with the lowest MIC values reported for *C. albicans* and *E. coli* ATCC 25922. The data obtained in this study supports the findings of the current study.

As seen from the results reported here, mint essential oil did not show any antibacterial activity against *E. coli* O157:H7. On the contrary, Singh et al. (2002), Karagözlü et al. (2011) and Moreira et al. (2005) reported that mint essential oil had an antimicrobial effect on *E. coli* O157:H7. This may have resulted from differences in methodology or concentrations of the essential oils used between the different studies.

The lack of any antimicrobial efficiency of cilantro, and chamomile essential oils tested in this study is in contrast to the results obtained by other authors (Delaquis et al., 2002; Roby et al., 2013). However, comparison of the data obtained in this study with previously published data is not easy, considering that the composition of plant oils and extracts vary according to environmental conditions and plant species (Sivropoulou et al., 1995).

Total phenolic content

Phenols are organic compounds that contain a hydroxyl group bound directly to the aromatic ring; the H-atom of the hydroxyl group can trap peroxyl radicals, preventing other compounds from being oxidized (Nguyen et al., 2003). In this way, the presence of phenols may contribute towards the antioxidant activity.

The results obtained in the current study showed that the total phenolic content (determined by the Folin-Ciocalteu method) varied between 2.33 and 695.06 mg GAE per 100 g among the fifteen essential oils tested (Tab. 3). Essential oil from dill showed the highest (695.06 mg GAE/100 g) amount of phenolic compounds followed by essential oils of mint (485.272 mg GAE/100 g), fennel (232.036 mg GAE/100 g), and flos lavandulae (flower) (60.217 mg GAE/100 g). Samples from hyssop (leaf), clary sage and pot marigold showed the lowest total phenolic content. Correlating with the phenolic content of 695.058 ± 2.12 mg GAE/100 g (see Tab. 3), the essential oil from dill also showed highest antioxidant activity.

According to Viuda-Martos et al. (2011), total phenolic content of fennel and lavender essential oils were 146.51 and 140.33 GAE/(mg/L), respectively. Dorman et al. (2003) reported the total phenolic content in different varieties of *Mentha* (Dill) to be about 128–230 mg/g of extract in GAE, and the main phenolic compounds found in extracts of *Mentha spicata* were eriocitrin, luteolin, rosmarinic acid, and caffeic acid. However, the amount of phenolic compounds found in the current study is lower than the reported amounts.

Flavonoids (e. g. quercetin, apigenin and hesperidin) and volatile oils can be obtained from chamomile, lavandulae, dill and fennel. These active ingredients produce varied pharmacological effects including anti-microbial, anti-diabetic, anti-inflammatory, anti-neoplastic, immuno-

TABLE 3: *Amounts of total phenolic compounds of essential oils.*

Essential oils	Total phenols GAE (mg/100 g)
Herba lippiae	31.679 ± 0.45
Anise	23.834 ± 0.56
Chamomile petal	31.057 ± 1.10
Clary sage	6.993 ± 0.80
Pot Marigold	8.562 ± 0.12
Dill	695.058 ± 2.12
Fennel	232.036 ± 1.22
Cilantro	28.822 ± 0.76
Hyssop (pedicle)	31.242 ± 0.48
Hyssop (flower)	22.629 ± 0.22
Hyssop (leaf)	2.334 ± 0.04
Flos Lavandulae (pedicle)	32.326 ± 0.75
Flos Lavandulae (leaf)	29.391 ± 0.34
Flos Lavandulae (flower)	60.217 ± 0.85
Mint	485.272 ± 0.94

GAE: Gallic acid equivalent; Results are given as mean ± standard deviation of triplicate experiments.

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regulatory effects as well as protection from liver damage (Yan et al., 2003). It is believed that phenolic compounds not only contribute to the quality and nutritional value of foods by modifying the colour, taste, aroma and flavour but also provide health beneficial effects. Through their contribution towards the antioxidant activity of plants (Rahiman et al., 2013), phenolic compounds serve in plant defence mechanisms to counteract reactive oxygen species (ROS) and subsequent damage at the molecular level as well as and damage from microorganisms, insects and herbivores (Vaya et al., 1997).

Antioxidant activity

Antioxidant activity is a complex process usually occurring through several mechanisms. Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts or pure compounds cannot be evaluated by a single method; rather, at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier et al., 2002; Aruoma, 2003). For this reason the antioxidant activity of the 15 different essential oils was determined by two spectrophotometric methods, DPPH and phosphomolybdenium assay.

The radical scavenging effects of essential oils were tested using a methanolic solution of the DPPH free radical, which shows a deep purple colour with the maximum absorption at 517 nm. Table 4 presents the radical scavenging capacity of the plant essential oils tested (expressed as EC_{50} values) using the "stable" free radical, DPPH. Butylated hydroxytoluene (BHT) was used as a positive control. The DPPH free radical has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Amorowicz et al., 2004).

The 15 essential oils used in this study exhibited varying degrees of scavenging capacities between 4.79 ± 0.14 % – 92.70 ± 1.12 %. The results showed that the most of the essential oil samples possessed low radical scavenging activity of less than 50 % DPPH. Essential oil from dill showed the strongest radical scavenging effect (92.70 ± 1.12 %), which was comparable to the positive control BHT (97.23 ± 0.48 %). This was followed by radical scavenging activities of essential oils from mint (81.00 ± 1.02 %), anise (71.53 ± 1.08 %), chamomile (38.26 ± 0.17 %), and flos lavandulae (pedicle) (32.84 ± 0.56 %). Essential oils from pot marigold and hyssop (flower) showed the lowest scavenging activity. Viuda-Martos et al. (2011) indicated that fennel and lavender essential oils inhibited the DPPH radical scavenging activity by 2.74 % and 4.11 %, respectively. However, these values are higher than what was found in the current study.

The lowest EC_{50} values were obtained with anise (23.14 µg/ml) and dill (35.19 µg/ml); these essential oils therefore can be classified as very strong and strong antioxidants, respectively. This was comparable to the antioxidant activity of the synthetic antioxidant BHT, which was within values obtained in other studies (Gourine et al., 2010; Borneo et al., 2009). In contrast, the remaining essential oils revealed poor antioxidant activity. The essential oils from the leaf and seeds of dill have been reported to possess good antioxidant activities (Delaquis et al., 2002; Kmiecik et al., 2001; Mohammad and Aburjai, 2004; Singh et al., 2005).

The antioxidant activities of essential oils were also investigated using the phosphomolybdenum assay and revealed similar results as the DPPH method. The data, expressed as ascorbic acid (AA) equivalents (mg AAE/g), are presented in Table 4. The antioxidant activities of the essential oils varied from 10.42–195.27 mg AAE/g. The highest antioxidant activity was obtained with essential oil from mint (195.27 mg AAE/g). It was followed by dill (186 mg AAE/g), anise (105.74 mg AAE/g) and flos lavandulae (pedicle) (63.39 mg AAE/g) whereas the remaining essential oils showed values below 25 mg AAE/g.

Zheljazkov et al. (2012) reported the antioxidant activity of hyssop essential oil as 2039 µmol of TE L^{-1}, whereas the antioxidant activity of lavender essential oil was determined to be 328 µmol of TE L–1. Zheljazkov et al. (2010) reported that the antioxidant activity of the essential oils from "Scotch" spearmint, "Native" spearmint, peppermint and Japanese cornmint were 4372, 1713, 1107, and 471 µmol of TE L^{-1}, respectively. Using the same method, the antioxidant activity of fennel and chamomile found in the current study was similar to the findings of Roby et al. (2013).

Conclusions

This study showed that essential oils of dill, hyssop (flower and leaf), hyssop, flos lavandulae (flower and leaf), mint, anise and fennel were effective in the growth inhibition of most pathogenic bacteria tested. Additionally, essential oils from mint, dill and anise revealed the strongest antioxidant activity. This was also correlated with the high total pheno-

TABLE 4: *Antioxidant activity of the essential oils and BHT (butylated hydroxytoluene) in DPPH (α, α-diphenyl-β-picrylhydrazyl) free radical scavenging activity and phosphomolybdenium assay methods.*

Essential oils	DPPH		Phosphomolybdenium assay
	% inhibiton	EC_{50} (µg/ml)	(mg AAE/g)
Herba lippiae	15.43 ± 0.65	nd	18.81 ± 0.65
Anise	71.53 ± 1.08	35.19 ± 0.41	105.74 ± 1.41
Chamomile petal	38.26 ± 0.17	nd	38.31 ± 0.81
Clary sage	15.53 ± 0.23	nd	24.16 ± 1.27
Pot Marigold	4.79 ± 0.14	nd	10.42 ± 0.25
Dill	92.70 ± 1.12	23.14 ± 0.85	186.98 ± 1.41
Fennel	24.92 ± 0.54	441.1 ± 0.25	42.33 ± 0.25
Cilantro	16.89 ± 0.39	nd	15.43 ± 0.09
Hyssop (pedicle)	26.79 ± 0.43	205.4 ± 1.41	55.76 ± 1.25
Hyssop (flower)	9.38 ± 0.13	nd	14.23 ± 0.41
Hyssop (leaf)	23.46 ± 0.24	nd	20.45 ± 0.54
Flos Lavandulae (pedicle)	32.84 ± 0.56	143.38 ± 1.82	63.39 ± 0.72
Flos Lavandulae (leaf)	27.007 ± 0.96	208.8 ± 0.51	33.39 ± 0.25
Flos Lavandulae (flower)	24.60 ± 0.55	nd	28.32 ± 0.41
Mint	81.00 ± 1.02	53.1 ± 0.32	195.27 ± 1.93
BHT	97.23 ± 0.48	19.55 ± 0.25	–

EC_{50} signifies concentration (µg/ml) for 50 % inhibition. Results are given as mean ± standard deviation of triplicate experiments. nd: not determinated. Antioxidant activity expressed as ascorbic acid equivalent (AAE).

lic content found in the essential oils of mint and dill. These plants are thus considered to be good sources of natural compounds with substantial antioxidant activity. The results of this study suggest the possibility of using essential oils or some of their components in foods to prevent the growth of food-borne bacteria and thus extend the shelf life of processed foods.

Conflict of interest

The authors declare that they have no conflict of interest.

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