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Chemical Composition and Antibacterial Effect of Medicinal Plants against Some Food-Borne Pathogens

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Abstract

Background: *Pulicaria gnaphalodes*, *Ducrosia anethifolia*, *Trachyspermum copticum*, *Foeniculum vulgare* Mill and *Majorana hortensis* Minch are widely used as herbal plants in traditional medicine and they have been reported to have a variety of therapeutic effects. This study was carried out to evaluate the antimicrobial effects of essential oils (EOs) extracted from these medicinal herbs against six species of food-borne microorganisms.

Materials and Methods: The EOs were analyzed by gas chromatography mass spectrometry (GC/MS). The detection of inhibitory effect of the EOs on the tested bacteria was carried out by agar disk-diffusion method and then MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of the EOs against six bacteria were determined.

Results: The analysis of the components of the essential oils (EOs) extracted by gas chromatography spectrometry allowed the identification of 63 compounds of the five tested EOs. All of these five tested EOs indicated an antimicrobial effect against strains of Bacillus sp and *Listeria Monocytogenes* ATCC1297. Essential oils from *T. copticum*, *M. hortensis* and *F. vulgare* possessed a wide spectrum of antibacterial activity against the growth of the six bacteria with zone diameter of inhibition (ZDI) between 14-32 mm, depending on the susceptibility of the tested organism.

Conclusion: Antibacterial efficacy shown by these plants provides a scientific basis and thus validates their use as medicinal remedies. Isolation and purification of different phytochemicals may further yield significant antibacterial agents.

Keywords: Essential oils; Antimicrobial activity; Chemical composition; Medicinal herb

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Introduction

In modern societies, the safety of meat and its products is widely recognized as meat consumption is important for human growth, development and maintenance of health (1). A major issue related to meat consumption is the presence of pathogens and among them the causative agents of food-borne diseases are the leading causes of illness and death (2). Food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food-borne illnesses outbreaks caused by the pathogens like *Staphylococcus aureus*, *Salmonella sp*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus sp*, and entero-pathogenic *Escherichia coli* (1,3). These bacteria cause over 90% of all cases of food poisoning (4). *Listeria monocytogenes* is a ubiquitous gram-positive pathogen responsible for a serious disease named listeriosis and the overall mortality rate associated with this disease is 30 %.

Antibiotic use has been banned in the European Union since January 2006. For this reason, scientists

have become interested in evaluating other alternatives to control specific microbial populations to modulate rumen fermentation (5). The antibacterial activities of spices and their essential oils have been known for a long time and a number of researches have reported the antibacterial effect of these spices, their essential oils as well as their derivatives (6). Plant essential oils are the potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microorganisms including food-borne pathogens (7). For a long time, plants from the Apiaceae family have been used as spices or drugs particularly due to their essential oils. A dozen important herbal medicinal products from this botanic family are used in some pharmacopoeias, antiseptic, expectorant, diuretic, carminative, vasodilator or spasmolytic actions (8). The members of this family are well known as vegetables, culinary and medicinal plants such as *Foeniculum vulgare*, *Ducrosia anethifolia Trachyspermum copticum* and etc (9).

Majorana hortensis Moench (Syn. *Origanum majorana* L.), commonly known as 'sweet marjoram', is a perennial aromatic herb of the family Lamiaceae (10). The aerial parts of the plant are used for the isolation of essential oils, which have a lot of uses in the flavor, perfumery and pharmaceutical industries. The essential oils are employed for external application in bruises, sprain, stiffs and paralytic limbs, toothache and as hot fomentation in acute diarrhea. In the food industry, it is mainly used as a spice in sausages but its use in the baked goods, the processed vegetables, condiments, soups, snack foods and gravies is also reported (11).

Pulicaria gnaphalodes (Vent.) Boiss. is an annual herb producing small bright yellow flowers (12). The chemical investigation of the *Pulicaria* genus has revealed the occurrence of molecules such as diterpenes, sesquiterpenes, caryophyllenes and caryophyllane derivatives and flavonoids (13). Various biological activities have been reported for some species of *Pulicaria*, such as antibacterial, antifungal and insecticidal properties (14).

The aim of the present study was to determine the chemical composition of some of the most important essential oils extracted from the cultivated plants all around Iran, including *Pulicaria gnaphalodes*, *Ducrosia anethifolia*, *Trachyspermum copticum*, *Foeniculum vulgare* Mill and *Majorana hortensis* Minch. We also aimed to evaluate the antimicrobial activity of these plants against pathogenic *Bacillus sp* and *Listeria Monocytogenes* ATCC1297. Data obtained from this study could help to identify the potential essential oils to be applied as food preservatives.

Materials and methods

Isolation of essential oil portions

Portions (100 g) of each prepared plant material were hydro distilled for 3h in a Clevenger type apparatus to isolate the essential oils. The obtained essential oils were dried over anhydrous sodium sulfate. After filtration, they were stored in the dark glass bottles at -4 °C until used for further analyses. The following oils were used: *Pulicaria gnaphalodes*, *Ducrosia anethifolia*, *Trachyspermum copticum*, *Foeniculum vulgare* Mill and *Majorana hortensis* Minch.

Gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) analysis

The composition of the volatile constituents was established by gas chromatography-mass spectrometry (GC-MS) Thermo-UFM (Ultra-fast model, Milan, Italy) equipped with flame ionization detector (FID) and a capillary column Ph-5 (nonpolar) (10 m × 0.1 mm, film thickness 0.4µm, the inner surface covered with stationary phase material, dimethyl siloxane phenyl 5 %). Helium was used as a carrier gas with inlet pressure of 0.5 ml/min. Column temperature was held at 60 °C for 3 min, programmed to 285 °C at the rate of 80 °C/min. Detector and injector chamber temperatures were 280 °C. GC-MS analyses were carried out on a Varian 3400 GC-MS system connected to a mass spectrometer model Saturn II, using an ion trap system having ionization energy of 70 eV, with a semi-polar DB-5 column, with dimensions of 30 m × 0.25 mm, and a film thickness 0.25µm. Gas pressure was 35 pounds per square inch, column temperature was 40 to 250 °C with a rate of increase of 4 °C/min, and the injection chamber temperature and the transfer line were set at 260 °C and 270 °C, respectively. The carrier gas was helium with a linear velocity of 31.5 cm/s. A scan time of 1 s with a mass range of 40-300 amu was used. The components of each oil were identified by comparison of their retention indices (RI) relative to n-alkenes (C7- C25) and confirmed by comparing their mass spectra with those of authentic samples or with data already available in the literature. The quantification of the volatile compounds was performed by GC-MS peak area normalization method using a Shimadzu C-R4AChromatopac without any correction factor (15).

Antimicrobial assay

Agar gel disk diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used in this study.

Microorganisms

Bacillus cereus, *Bacillus sphericus*, *Bacillus antheracoid*, *Bacillus coagulance*, *Bacillus subtlis*

and *Listeria monocytogenes* ATCC 1297 were obtained from the School of Veterinary Science of Shiraz University.

Disk diffusion susceptibility

Antibacterial Susceptibility Assay Muller-Hinton Broth (MHB, Merck) medium was used to grow the test isolates for 22 h at 37 °C. Final bacterial numbers were standardized to 1×10^6 cfu/ml. A total of 0.1 ml of bacterial suspension was poured into each plate containing Muller-Hinton Agar. The surface culture was prepared by sterile L shape pipet Pasteur and allowed to remain in contact for 1 min. Thereafter, a 5% concentration of each plant essential oils was prepared. The sterile filter paper disks (6-mm diameter) were placed on the cultures and 24 h after incubation at 37 °C the zone diameter of inhibition (ZDI) was measured in mm. In order to determine the sensitivity of each bacterial species tested, tetracycline was used as a positive control standard. All the experiments were performed in triplicate (1).

Determining the MIC and MBC

For each essential oil, a set of 9 sterile test tubes was used. The stock solutions (500 mg/ml) were further diluted in a 2-fold serial dilution to obtain the following concentrations: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.91, 1.95, and 0.98 mg/ml. One test tube as a negative control and tetracycline as a Positive control was used. An aliquot of 1ml of the bacterial suspension was inoculated into each tube. The negative control tubes were inoculated with the same quantity of extracts. All tubes were incubated at 37 °C for 24 h. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration (MIC). The contents of all tubes that showed no visible growth were cultured on Muller Hinton agar and incubated at 37 °C for 24 h. The MIC was considered as the lowest concentration that could not produce a single bacterial colony and the MBC was defined as the lowest concentration of the extract at which 99.9% of the inoculated microorganisms were killed (16).

Statistical analysis

In order to determine whether there is a statistically significant difference among the obtained results from zone inhibition assays, MIC and MBC average analyses were carried out using the SPSS V16.0 statistical software package. The differences between any means were tested by the Duncan test and the results were considered significant when $P<0.05$.

Results

Chemical composition of the EOs

The main constituents of the studied essential oils are presented in Table 1.

Table 1. Chemical composition (%) of identified compounds in the tested essential oils determined by gas chromatography- mass spectrometry.

Plant	Compounds	(%)	RI	RT(min)
T. copticum	β-Pinene	0.63	989.52	1.5
	Myrcene	0.40	1010.10	1.52
	p-Cymene	22.45	1049.25	1.6
	8-terpinen	35.45	1081.93	1.67
	Thymol	38.97	1309.99	2.11
F. vulagre	α-Pinene	0.76	956.99	1.42
	β-Pinene	0.55	989.52	1.5
	p-Cymene	0.34	1044.47	1.59
	Limonene	6.06	1058.73	1.62
	Fenchone	11.15	1121.99	1.76
	Methyl Chavicol	3.79	1232.62	1.96
	e-Anethole	76.80	1324.80	2.14
M. hortensis	β-Pinene	2.46	989.52	1.5
	Limonene	0.29	1058.73	1.62
	1,8 cineole	0.94	1063.43	1.63
	Linalool	86.44	1113.27	1.74
	geranyl acetate	1.17	1436.12	2.32
	β-elemene	1.60	1477.11	2.39
	e-caryophyllene	0.61	1488.60	2.41
	8-cadinene	1.49	1561.04	2.54
	1,1-di-epi-cubinol	0.39	1693.50	2.72
	ten-cadinol	2.85	1712.91	2.75

D. anethifolia	α-Pinene	3.09	956.99	1.42
	β-Pinene	0.63	989.52	1.5
	Limonene	0.51	1058.73	1.62
	Fenchone	0.29	1121.99	1.76
	Camphor	0.88	1172.33	1.88
	Decanal	0.48	1221.85	1.94
	Cis-Chrysanthenyl *acetate*	72.28	1289.91	2.07
	e-Anethole	4.33	1314.95	2.12
	trans-Pinocarvyl acetate	2.11	1329.69	2.15
	Carvacrol	2.52	1339.41	2.17
	Cis-Pinocarvyl acetate	0.46	1372.72	2.24
	Aromadendrene	1.19	1471.33	2.38
	α- Humulene	0.37	1488.60	2.41
	β-eudesmol	8.79	1680.44	2.7
P. gnaphalodes	α-Pinene	1.62	956.99	1.42
	p-Cymene	1.66	1054.01	1.61
	Limonene	1.80	1058.73	1.62
	1,8-cineole	14.12	1063.43	1.63
	Terpinolene	0.26	1095.52	1.7
	Linalool	1.13	1108.87	1.73
	cis-p-menth-*2*-en-*1-ol*	2.87	1130.61	1.78
	Chrysanthenone	1.98	1151.76	1.83
	Cis-*chrysanthenone*	2.56	1176.38	1.89
	Terpinen-4-ol	4.25	1221.85	1.94
	α-Terpineol	5.79	1232.62	1.96
	Nerol	0.53	1259.05	2.01
	Thymol	0.30	1294.96	2.08
	Carvacrol	4.54	1314.95	2.12
	α-cubeben	3.21	1363.31	2.22
	β-cubeben	1.67	1424.19	2.3
	germacrene-D	1.79	1528.08	2.48
	Alpha-murolene	3.08	1544.66	2.51
	8-cadinene	10.98	1566.46	2.55
	Trans-Calamene	0.75	1582.58	2.58
	α-calacorene	1.58	1613.66	2.6
	Elemicine	1.33	1633.96	2.63
	1,10-epi-cubenol	1.24	1693.50	2.72
	1- epi-cubenol	1.09	1700	2.73
	Ten-Cadinol	9.30	1712.91	2.75
	G-Cadinol	13.76	1725.73	2.77
	α-Cadinol	0.97	1744.80	2.8

The analysis of the components by gas chromatography and gas chromatography mass spectrometry allowed the identification of 63 compounds, accounting for 94.16%, 97.93%, 97.9%, 99.45% and 98.24% of the composition of volatile substances of *Pulicaria gnaphalodes*, *Ducrosia anethifolia*, *Trachyspermum copticum*, *Foeniculum vulgare* Mill and *Majorana hortensis* Minch, respectively. The main components of *T. ammi* EO were thymol (38.97%) and 8-terpinene (35.45%). The components of *F. vulgare* Mill EO were E-anethole (76.80%) and Fenchone (11.15%). The major components of EO obtained from *M. hortensis* Minch was linalool (86.44%). The main components of *D. anethifolia* were Cis-chrysanthenyl acetate (72.28%) and E-anethole (4.33%). 1, 8-Cineole (14.12%) and G-cadinol (13.76%) were the major constituents of *P. gnaphalodes* EO.

Antimicrobial activity of the tested EOs

For evaluation of bacterial susceptibility to herbal agents three standard tests were carried out, including disk diffusion assay, minimum inhibitory concentration and minimum bactericidal concentration. As can be seen in Tables 2-4, the *T. copticum* essential oil reveals the potential antibacterial activity against all of the tested bacteria with the mean ZDI ranging from 23.66 to 32.66 mm. The *Listeria monocytogenes* showed the most susceptibility to the essential oil of *T. copticum* with the ZDI of 32.66 mm that was more than tetracycline with the ZDI of 30.5 mm (Table 2). As have been shown in Tables 2-4, the lowest effect of essential oil against bacteria was related to *D. anethifoli*a and there was no effect on *B. subtlis* and *B. coagulance*. The ZDI of *T. copticum* essential oil against all of the bacteria was more than control group (tetracycline).

Table 2. The inhibition zone (mm) of selected herbal essential oils against food-borne pathogen bacteria.

Herbal (EO)	*B. cereus*	*B. sphericus*	*B. anheracoid*	*B. coagulance*	*B. subtlis*	*L. monocytogenes*
T. copticum	31.33±1.15^{c}	30±2^{c}	29±1.73^{b}	27.66±3.78^{c}	23.66±0.57^{bd}	32.66±6.42^{cd}
F. vulgare	15.66±3.51^{b}	15.33±3.05^{ab}	14±3.46^{a}	15.33±2.30^{b}	16.66±4.16^{b}	16±3^{a}
P. gnaphalodes	0^{a}	15.33±1.15^{ab}	14±2^{a}	0^{a}	0^{a}	12±1^{a}
D. anethifolia	13.33±3.05^{b}	11.33±1.15^{a}	17±5^{a}	0^{a}	0^{a}	19.66±3.21^{ab}
M. hortensis	15.33±3.05^{b}	20±4^{b}	16±2^{a}	14±3.46^{b}	20.66±4.16^{bc}	26±2^{bc}
Tetracycline	18±4^{b}	35.33±6.42^{c}	29±2^{b}	26.66±4.61^{c}	18±3.46^{b}	30.66±5.13^{cd}

Values are mean ± S.D. of three replicates. Means with different superscripts within the same row are significantly different ($p<0.05$).

The MIC and MBC values of the leaf essential oils, at different concentrations ranging from 0.98 mg/ml to 250 mg/ml, in comparison with the activity of tetracycline has been shown in Tables 3 and 4.

Table 3. MIC (mg/ml) values of essential oil of the selected plants against isolated bacteria.

Herbal (EO)	*B. cereus*	*B. sphericus*	*B. antheracoid*	*B. coagulance*	*B. subtlis*	*L. monocytogenes*
T. copticum	1.95	1.95	1.95	1.95	3.91	0.98
F. Vulgare	7.8125	7.8125	7.8125	7.8125	7.8125	7.8125
P. gnaphalodes	125	7.8125	7.8125	125	125	15.625
D. anethifolia	7.8125	15.625	7.8125	125	NO	3.91
M. hortensis	7.8125	3.91	7.8125	7.8125	3.91	1.95
Tetracycline	7.8125	0.98	0.98	1.95	3.91	0.98
Control negative	NO	NO	NO	NO	NO	NO

NO: No effect
EO: Essential oil

The results of the MBC method (Table 4) are consistent with the results of the disk diffusion test as shown in Table 2. In summary, the essential oil extracted from *T. copticum* showed the highest antibacterial activity for these bacteria while *D. anethifoliae* essential oil had the lowest antibacterial activity.

Table 4. MBC (mg/ml) values of essential oil of the selected plants against isolated bacteria.

Herbal (EO)	*B. cereus*	*B. sphericus*	*B. antheracoid*	*B. coagulance*	*B. subtlis*	*L. monocytogenes*
T. copticum	3.91	3.91	3.91	7.8125	7.8125	3.91
F. Vulgare	15.625	15.625	31.25	15.625	15.625	15.625
P. gnaphalodes	NO	31.25	62.5	NO	NO	31.25
D. anethifolia	31.25	31.25	15.625	NO	NO	15.625
M. hortensis	15.625	15.625	15.625	31.25	7.8125	3.91
Tetracycline	15.625	1.95	3.91	3.91	7.8125	3.91
Control negative	NO	NO	NO	NO	NO	NO

NO: No effect
EO: Essential oil

Discussion

The results of the essential oils extracted from different fresh aromatic plants collected from Borazjan, a city located in Bushehr Province in the south of Iran, have been shown in Table 1. To date, a large number of studies have focused on *T. copticum* EO and some of them have reported thymol as the main compound (17,18). In the present study, the major components of *T. copticum* EO obtained from Iran were thymol (38.97%) and 8-terpinen (35.45%). Similar to our results, Moazeni et al. (2012) reported that the essential oils extracted from Iran ecotypes consisted of 50.07% thymol (19). It is clear that geographical variation, cultivar differences, the stage of plant growth, preparation process and other factors may influence oil composition both quantitatively and qualitatively (20). Acidic nature of the hydroxyl group in thymol and the involvement of the hydroxyl group in the formation of hydrogen bonds may explain the highest antimicrobial activity (21). Based on current evidence, *T. copticum* EO can inhibit food-borne pathogenic microorganisms such as *Staphylococcus aureus* (22), *Pseudomonas aeruginos* (23), *Salmonella sp, Bacillus subtilisu, Aspergillus flavus and Escherichia coli* (24). In the present study, *T. copticum* EO had the strongest antibacterial effect against *L. monocytogenes* (32.66 mm inhibition zone). Detailed data obtained from a previous study indicated that the growth of pathogenic *Bacillus cereus* (35 mm inhibition zone) was significantly affected by *T. copticum* EO (24).

Pulicaria gnaphalodes is traditionally used as a flavoring agent in food. The major components of the essential oil of *P. gnaphalodes* obtained from different areas in Mashhad, a city located in Iran, were completely different from our results (13). Also, *B. sphericus* was more sensitive to *P. gnaphalodes* with the ZDI of 15.33 mm in comparison to *B. antheracoid* with the ZDI of 14 mm. It has further been reported that the MIC values of *P. gnaphalodes* against *Salmonella typhimurium* and *Staphylococcus aureus* were 0.2 and 0.1 v/v, respectively (25).

Verma et al. (2010) showed that *M. hortensis* has two chemotypes, including terpinen-4-ol and cis-Sabinene hydrate (26). In a research conducted by Verma et al. (2010) the main constituents of *M. hortensis* EO were Z-Sabinene hydrate (31.81), terpinen-4-ol (22.02) and (E)-Sabinene hydrate (27). The main components of the EO in the present study were Linalool (86.44%), ten-cadinol (2.85%) and β-pinene (2.46%). These differences in chemical compositions of the oils could be attributed to the effect of environment on the plants (28). Investigations showed that *M. hortensis* EO can inhibit food-borne pathogenic microorganisms such as *Staphylococcus aureus* (22 mm) and *Enterobacter spp* (15 mm) (29). *M. hortensis* showed that EO was more active than *P.gnaphalodes* against all tested pathogenic bacteria. The higher antibacterial activity of *M. hortensis* is possibly related to the high amount of Linalool that has been reported to possess antibacterial properties.

The components of *F. vulgare* EO in our study were e-Anethole (76.80%), Fenchone (11.15%) and Limonene (6.06%) which is relatively similar to *F. vulgare* composition from Pakistan (30). Dua et al. (2013) reported that the ZDI of *F. vulgare* against *Staphylococcus aureus* and *Bacillus pumilus* were 11.17 and 12.67 mm, respectively (31). The growth ZDI in the presence of *F. vulgare* EO seeds was 15.66 mm for *B. cereus*, 15.33 mm for *B.sphericus*, 14 mm for *B. antheracoid*, 15.33 mm for *B. coagulance*, 16.66 mm for *B. subtlis* and 16 mm for *L. monocytogenes*. However tetracycline was more efficient with the growth ZDI of 18-35.33 mm.

N-decanal (70.1%), α-pinene (12.4%) and dodecanal (5.4%) are vastly utilized in the industrial companies as major components of essential oils. N-Decanal is used in fragrances and flavorings (32). The main components of *D. anethifolia* in this study were cis-chrysanthenyl acetate (72.28%), β-eudesmol (8.79%), and e-Anethole (4.33%). There is also an interesting similarity between our study and the study conducted by Sohrabi et al. (2016) regarding two main components including 1, 8-cineole 14.12% and G-Cadinol 13.76% (33). Biological activities such as antimicrobial, antibacterial and antianxiety effects have been reported for *D. anethifolia* (34). The growth ZDI in the presence of *D. anethifolia* EO seeds was 13.33 mm for *B. cereus*, 11.33 mm for *B. sphericus*, 17 mm for *B. antheracoid*, 0 for *B. coagulance*, 0 for *B. subtlis* and 19.66 mm for *L. monocytogenes*, although tetracycline showed to be more efficient with the growth ZDI of 18-35.33 mm.

Conclusion

In conclusion, the essential oils extracted from medicinal plants in this study have potential antibacterial activities against the bacteria strains. Our results support the use of these plants in traditional medicine and suggest that some of the plant essential oils possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.

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Author Contributions

The present study was funded by HH and NGH. HH and NGH were also involved in the collection of data, statistical analysis and drafting of the manuscript. Gas chromatography (GC) analysis well done by MA K. MAKand FE read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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