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Analysis of Chemical Compounds and Antibacterial Effect of Five Medicinal Plant Essential Oils on Infectious Bacteria

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Abstract

Fundamental research on plants in order to identify their pharmaceutical agents and their effects on pathogens has been increased in medicinal plant research centers around the world, especially in Iran. This study has been conducted to determine the antibacterial effects of *Pulicaria gnaphalode*, *Ducrosia anethifolia*, *Trachyspermum copticum*, *Foeniculul vulgare* Mill, and *Majorana hortensis* Minch essential oils on *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Shigella boydii*. Their essential oils were extracted by a Clevenger apparatus following preparation of plants powder in appropriate condition. Gas chromatography-mass spectrometry (GC-MS) was used for essential oil (EOs) chemical analysis. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also investigated for studying the EO effects on bacteria. Sixty three compounds were identified in the EO analysis of five plants by GC-MS. All EOs tested in this study showed antibacterial properties on all three bacterial strains. EO of the Trachyspermum copticum showed stonger antibacterial effects than the other herbs. It is notable that there was a significant difference between the antibacterial activities of the EOs. The results of this study provide a scientific basis for using these plants in traditional home remedies. On the other hand, extraction and purification of these EOs can provide more phytochemicals with stronger antibiotic properties.

Keywords: Anti-bacterial effect, Essential oil, Minimum Bactericidal Concentration, Minimum Inhibitory Concentration.

1. Introduction

Antibiotic resistance is the main problem during bacterial infection treatment. Antibiotic resistant bacteria cause higher rates of mortality in comparison to other pathogenic bacteria (1). Enterobacteriacea, *Pseudomonas*, and *Acinobacter* Spp. are Gram-negative and *Staphylococcus*, *Streptococcus*, and *Enterococcus* Spp. are Gram-positive antibiotic resistant bacteria that cause nosocomial infections (2, 3). Efforts to find new therapeutic strategies have been increased nowadays due to the increase in antibiotic resistance (4). One of the most promising strategies is the use of medicinal plants that are highly compatible with the human body.

Proteus vulgaris, *Pseudomonas aeruginosa*, and *Shigella boydii* are Gram-negative bacteria from Enterobacteriaceae (5-7). *Proteus vulgaris* and *Pseudomonas aeruginosa* are the main cause of nosocomial infections (8, 9). *Proteus vulgaris* is one of the normal flora of the human digestive system that can cause urinary tract infection, especially in patients with weak immune system.

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This pathogen can also cause infection in animals such as cat and dog (8). In recent years, antibiotic resistance has been spread rapidly in these bacteria, which has increased the costs of treatment, mortality risk in patients, and threats to public health (10). *Pseudomonas aeruginosa* has been associated with difficulty in avoiding infection during chemotherapy (9). *Shigella causes* bloody diarrhea in humans (11). More than 165 million cases of illness and one million deaths per year have been reported by *Shigella* (6).

Pulicaria gnaphalodes is a member of the Asteraceae family, which include 200 species and 2000 genus. It is mostly found in Asia, Europe, and Africa (12, 13). Its height is 10-30 cm, with golden yellow flowers that grow in sandy places (14). According to available studies, this plant contains compounds such as flavonoids, thymol, benzoic acid, steroids, terpenes and phenolic compounds (12, 14). Flavonoids in this plant are effective as benzodiazepine receptor ligands in epilepsy treatment (15).

Ducrosia anethifolia is a perennial *herbaceous* plant from the Apiaceae family, which is native to Iran, Pakistan, and Afghanistan (16, 17). N-decanal, α-pinene, and dodecane are the main components of the EO extracted from this plant (16-18). Antimicrobial, antimicrobial, and anti-anxiety activities have been reported for this plant (19). Previous studies have shown a significant inhibitory effect of *D. anethifolia* on a number of *Mycobacterium* species (20).

Trachyspermum copticum is a member of the Apiaceae family, which is found mostly in India, Iran, and Egypt (21, 22) and has small white flowers with brown beans (23). The EO of this plant contains gamma-terpinene, p-cement, α-pinene, β-pinene, and other compounds (23, 24). These compounds can degrade bacteria by penetrating into the bacterial cell wall and membrane (25). *T. copticum* antibacterial effect on *Salmonella typhimurium* and *Staphylococcus aureus* was determined in the previous studies (26).

Foeniculum vulagre Mill is an Apiaceae family member and native to India, Egypt, and other countries, which is used for its leaves and seeds (27). Various studies have been done on the antimicrobial activity of its EO and extract (28, 29). Phenolic compounds in fennel can inhibit free radicals and act as antioxidants (30). The effect of this plant on a group of Gram-positive bacteria such as *Clavibacter, Rhodococcus* and Gram-negative ones, such as *Pseudomonas viridiflava* has been identified in the previous studies (31).

Majorana hortensis Minch is a perennial herb of the Lamiaceae family, which is native to Cyprus and the eastern Mediterranean, whose aerial parts are used for their essential oils (32, 33). Various biological activities, such as anxiolytics, anticonvulsants, anti-diabetes, antimicrobials, anti-ulcer, and anti stomach ulcer have been reported for this plant (32, 34, 35). Thymol in its essential oil causes the loss of balance in the cell membrane of the bacteria and fungi and causes them to die (25). In the previous studies, the antibacterial effect of this plant on S*taphylococcus aureus*, *S. coagulase* negative bacteria was determined (35).

2. Materials and methods

2.1 Preparation of herbal samples

The aerial parts of plants were collected from their natural habitat in Bushehr province, Iran. Samples were dried out in a dry and dark environment (far from sunlight). Samples were also pulverized to obtain a powder.

2.2. Essential oil extraction

600 ml distilled water was added to 50 g powder in a round-bottom flask. The EOs were extracted using a Clevenger apparatus and stored at -4 °C.

2.3. Essential oil analysis

EOs were analyzed by Gas chromatography (Thermo-UFM) equipped with flame ionization detector (FID) and PH-50 capillary column (10m length, 0.1mm internal diameter, 0.4 mm film thickness). The temperature of the detector and the injection chamber was 280 °C. Temperature range was from 60 to 280 °C, which was increased 80 °C each min. Samples remained at favorite temperature for three min. Helium gas with a flow rate of 0.5 $mm.min^{-1}$ was used as carrier gas (moving phase).

2.4. Antibacterial activity by disc diffusion method

Proteus vulgaris, Pseudomonas aeruginosa, and *Shigella boydii* were used to study the antibacterial properties of plants. The antibacterial activity of plants was evaluated using disc diffusion method. The discs were sterilized by UV hood. The bacterial suspension was cultured on agar medium. The prepared disks were then placed in a bacterial culture medium. Tetracycline disk was used as a control in this study. Mediums then were incubated at 37 °C for 24 h. A zone of inhibition diameter was reported in ml (three replicates per plant were considered).

2.5. Minimum Inhibitory (MIC) and Minimum Bacterial Concentration (MBC)

Serial dilution method was used to deter-

Table 1. Chemical compounds of essential oil and their amounts in tested plants analyzed by GC-MS.

Plant	Compounds	(%)	RI	RT(min)
Trachyspermum 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
Foeniculum vulagre Mill	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
Majorana hortensis Minch	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
Ducrosia 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

Continued Table 1.

Pulicaria 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

mine the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC). 0.5 ml sterile tryptic soy broth (TSB) medium was added to 11 test tubes. 0.5 ml plant extraction (20%) was added to the first test tube of each row. Then, 0.5 ml of the first test tube solution was added to the next one. This continued until the last test tubes. 0.5 ml of the last test tube solution was also discarded. In the next step, 0.5 ml bacterial suspension (106 units) was added to each test tube. Negative (medium+bacteria) and positive (medium+plant extract) controls were also considered for this test. Then the tubes were incubated for 24 h at 37 °C. Based on the MIC definition, the last tube with no turbidity was considered as MIC. To determine the MBC, the solution of the transparent tubes of the previous step was cultured in the tryptic soy agar (TSA) medium. A plate with no bacterial growth was considered as MBC.

3. Results

Based on the results of GC, the most important compounds of T. copticum EO were thymol (38.97%), terpinene (35.45%), and p-cymene (22.25%). Anethole (76.80%) and Finchon (11.15%) were the main compounds of fennel (*F. vulagre*) EO. Other EO compounds in samples are shown in Table 1.

The results of this study showed the antibacterial effects of some used EOs on *P. vulgaris, P. aeruginosa*, and *S. boydii*. It is notable that the antibacterial effect of these EOs is comparable to the common antibiotics used against these bacte-

Table 2. Comparison of the average antibacterial activity of the tested plants in millimeters.

Bacteria	Antibiotic and tested plants EO					
	Tetracyclin	***M. hortensis***	***T. copticum***	***F. Vulgare***	***P. gnaphalodes***	***D. anethifolia***
P. vulgaris	32.6±4.6bc	22±3.6a	34.3±2c	21±3.6a	24.6±5a	26.6±3b
S. boydii	32.3±2.5d	2±3bc	3±2d	17.3±3b	23.3±4.1c	8.3±1.5a
P. aeruginosa	33.3±4.1c	11±1a	29.6±1.5a	11.6±1.5a	21.3±3b	11±1a

Diameter of inhibition zone±SD.

The values represented by the same letters were not significant at $P>0.05$.

Non-similar alphabets in each row indicate a significant difference between treatments.

ria. The diameter of inhibition zone of tetracycline for *P. vulgaris, P. aeruginosa*, and *S. boydii* were 32.6, 33.3, and 23.3 mm, respectively. According to the previous studies, the antibacterial effect of EOs was considered high, moderate, and no activity if the diameter of inhibition zone were ≥15 mm, 10-15 mm, and ≤15 mm, respectively (36). Our results indicated that all five plants had high antibacterial effects on *P. vulgaris*. *P. aeruginosa* was highly affected by *P. gnaphalodes, T. copticum*, and *M. hortensis*. It is notable that all the tested medicinal plants had a high antibacterial effect on S. boydii, except *D. anethifolia* (Table 2). The MIC and MBC results can be seen in table 3. These results indicate that the EO of *T. copticum* had the highest antibacterial effect on the studied bacteria.

4. Discussion

In recent decades, the research priority has dropped to make new and effective drugs, while the world faces pathogens with drug resistance. One of the main concern in this regard is the cost of treating drug-resistant infections due to the high cost of new effective drugs and long duration of treatment period compared to the susceptible bacterial infections. The antimicrobial activity of plants is generally due to the presence of phenolic compounds, saponins, tannins, and flavonoids in their structures. These compounds have antimicrobial properties by affecting the cell membrane of microorganisms, or by inhibiting the structural enzymes of their cell membranes (37).

In this experiment, *T. copticum* EO had the greatest impact on the tested bacteria. The strongest effect of this extract was on *P. vulgaris* with 34.3 mm zone of inhibition, which was more than the tested antibiotic. Gandomi *et al*. (38) and Rasooli *et al*. (39) showed that *Thymol* (39.38%) is the main compound of *T. copticum* EO. Our results also indicated that thymol (38.97%), terpinene (35.45%), and p-cymene (22.25%) were the main compounds of this plant EO. Similar to our results, Moazeni *et al.* showed thymol as the main compound of this plant EO (40). The antibacterial effect of *T. copticum* on *S. typhimurium* and *S. aureus* was confirmed in the previous studies (26). Kavoosi *et al.* determined the inhibitory effect of *T. copticum* on *Escherichia coli, Salmonella typhi*, and *Bacillus subtilis*, which is likely affected by thymol. *Thymol* can destroy the cell membrane of bacteria (41). The inhibitory effect of this plant

Table 3. MIC and MBC of tested plants EO

	MBC			**MIC**		
	P. aeruginosa	***S. boydii***	***P. vulgaris***	***P. aeruginosa***	***S. boydii***	***P. vulgaris***
D. anethifolia	No	No	1.562	6.25	25	0.39
P. gnaphalodes	1.562	1.562	0.78	0.78	0.78	0.39
F. vulgare	25	6.25	1.562	6.25	1.562	0.78
T. copticum	0.78	0.78	0.39	0.195	0.195	0.195
M. hortensis	25	3.125	1.562	6.25	0.78	0.78
Tetracyclin	0.39	0.78	0.39	0.195	0.195	0.195
Negative Control	No	No	No	No	No	No

on *Proteus mirabilis* and *Citrobacter freundii* was also indicated by Kazemi *et al*. (42).

Linalool is the main compound of the *M. hortensis* EO. Its antibacterial effect is due to linalool and thymol, which damage the bacterial cell membrane. In the previous studies, the antibacterial effect of this plant on *Staphylococcus aureus*, *S. coagulase* negative, *Enterobacter* spp, *Proteus* spp, *Acinetobacter* spp, and *Klebsiella* spp was determined (35). Our results indicated the high antibacterial effect of this EO on all the tested bacteria (zone of inhibition ≥15 mm). Kozlowska *et al.* showed the effect of *M. hortensis* on *Acinetobacter baumannii* ATCC 19606 previously (34). The results of MIC and MBC showed the high effect of this plant on the tested bacteria.

Cis-Chrysanthenyl acetate (72.28%) and β-eudesmol (8.79%) were the main compounds of *D. anethifolia* EO. There is a significant similarity between the findings of our study and Sohrabi *et al.* results for the combination of Cis-Chrysanthenyl acetate and β-eudesmol (43). These results indicated that the effect of *D. anethifolia* EO on microorganisms is related to the monoterpene compounds of their secondary metabolites. The results of this study did not show any antibacterial effect of *D. anethifolia* on *S. boydii* (zone of inhibition ≤10 mm), but it had a significant antibacterial effect on other tested bacteria in comparison to the control antibiotic. The diameter of inhibition zone was 11 mm and 26.6 mm for *P. aeruginosa* and *P. vulgaris*, respectively. *D. anethifolia* had the highest effect on *P. vulgaris* based on MIC and MBC data. The previous studies also showed the antibacterial effect of this medicinal plant on various species of *Mycobacterium* (20).

Phenolic compounds in fennel can inhibit free radicals and act as an antioxidant (30). EO analysis of the *F. vulagre* showed the highest concentration for anethole. Given thatthis compound can damage the bacterial cell membrane, anethole may be the main reason for the inhibitory effect of this plant on *P. vulgaris*, *P. aeruginosa*, and *S. boydii*. Other reports have also indicated the antibacterial effect of this plant on Gram-positive (*Clavibacter* and *Rhodococcus*) and Gram-negative (*Pseudomonas viridiflava*) bacteria (31). Anwar *et al*. reported the inhibitory effect of the fennel EO on *E. coli* and *B. subtilis* (30).

Cineole was the main compound of *P. gnaphalodes* EO. It is notable that our result about the compounds of *P. gnaphalodes* EO was completely different from Bashi *et al.* (13). In this experiment, *P. gnaphalodes* had a significant effect on the three bacteria and showed a significant difference with the control antibiotic. The inhibition zone diameter by *P. gnaphalodes* EO was 24.6, 21.3, and 23.3 mm for *P. vulgaris*, *P. aeruginosa*, and *S. boydii*, respectively. It is notable that the diameter of the inhibition zone by tetracycline on the three bacteria was 32.6, 33.3, and 32.3 mm, respectively.

5. Conclusion

According to the results of this study, it can be stated that the essential oils of *Pulicaria gnaphalodes, Ducrosia anethifolia*, and *Trachyspermum copticum* have high antibacterial effects *in vitro*. It is suggested that more studies (especially *in vivo*) be done to determine the effective dose, main antibacterial compound, and action mechanism of these plant EOs to introduce them as a new antibacterial agents.

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Conflict of Interest

None declared.

6. References

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