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Original Article

Chemical Composition and Antimicrobial Effect of Five Essential Oils on Pathogenic and Non-Pathogenic *Vibrio parahaemolyticus*

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ABSTRACT

Background and Objectives: *Vibrio parahaemolyticus* is the causative agent of gastroenteritis due to consumption of contaminated seafood. The aim of the present study was to determine the chemical composition of Essential Oils (EOs) of five plants (*Artemisia absinthium, Zataria multiflora Boiss., Pulicaria gnaphalodes, Trachyspermum ammi* and *Cuminum cyminum*) and to evaluate their antimicrobial activity against pathogenic and non-pathogenic *V. parahaemolyticus*.

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

Results: The analysis of the components of the EOs by gas chromatography mass spectrometry allowed the identification of 76 compounds. Of the five tested EOs, four oils exhibited an antimicrobial effect against both strains of *V. parahaemolyticus*. In all EOs tested, Pathogenic *V. parahaemolyticus* showed more sensitivity than non-pathogenic *V. parahaemolyticus*. The strongest EO against pathogenic *V. parahaemolyticus* were *T. ammi* with 63.42% thymol and *C. cyminum* with 29.02% cuminaldehyde and 20.70% α-terpinene-7-al, equally (31 mm inhibition zone). Non-pathogenic *V. parahaemolyticus* showed the most sensitivity against *Z. multiflora* Boiss. EO with 73.64% carvacrol (27 mm inhibition zone and the lowest MIC (0.025%) and MBC (0.05%)). Despite the large amount of thujone, *A. absinthium* EO in this study did not show antibacterial activity in disk diffusion assay, MIC or MBC values.

Conclusions: The results of this study suggest that *Z. multiflora* Boiss. and *T. ammi* have strong antimicrobial activity against both pathogenic and non-pathogenic *V. parahaemolyticus.*

Keywords: Chemical Composition, Antimicrobial Effect, Essential Oils, *Vibrio parahaemolyticus*

Introduction

There has recently been considerable concern with the increasing incidence of food-borne diseases, which have become a relevant public health issue (1). In spite of the advances in the sanitation techniques, inspection services and improvements in the cold distribution chain, the contamination of foods with undesirable microorganisms is a potential risk during food processing, further processing, storage and distribution (1).

Vibrio parahaemolyticus is a halophilic Gram negative bacterium that causes acute gastroenteritis in humans. During 1973–1998, 40 outbreaks of *V. parahaemolyticus* infections were reported to the CDC and these outbreaks included more than 1000

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illnesses (2). Food poisoning caused by this pathogen is generally associated with the consumption of raw or undercooked seafood, although the infection is associated with poor hygiene in cooked seafood. An increase in the incidence of infection may be predicted as a consequence of climate change (higher water temperatures), increasing salinity in some waters and by a general shortage of clean water for washing (3). Most clinical strains of *V. parahaemolyticus* possess a major virulence factor, the thermostable direct hemolysin (TDH) which exhibit β-hemolysis on Wagatsuma agar. Clinical manifestations include diarrhea, abdominal cramps, nausea, vomiting, headache, fever and chills, with incubation periods from 4 to 96h (4).

More cultivated communities prefer to consume foods without synthetic additives (5). Plant essential oils have been used for centuries in food preservation, yet there are potential sources of novel antimicrobial compounds with different modes of action. Also, consumers are used to the presence of spices in food products which are mainly used to improve palatability (6). But sometimes effective antimicrobial doses may adversely affect organoleptic properties (7). So, accurate knowledge of the minimum inhibitory concentrations of essential oils is necessary (7). Snoussi *et al.* (2008) have tested the anti vibrio spp. activity of five Tunisian essential oils extracted from *Mentha longifolia*, *Mentha pulegium*, *Rosmarinus officinalis*, *Eugenia caryophyllata* and *Thymus vulgaris*. These authors reported that *T. vulgaris* and *E. caryophyllata* oils exhibited a high degree of anti vibrio spp. activity, especially against *V. parahaemolyticus* strains with a diameter of zone growth inhibition ranging from 14.66 to 28 mm. They reported that MIC and MBC values were interestingly low for thyme oil (MIC 0.007–0.015% w/v) and (MBC > 0.031–0.125% w/v) (8). Some studies have been performed on the composition of *Artemisia absinthium* (9), *Zataria multiflora Boiss.* (10), *Pulicaria gnaphalodes* (11), *Trachyspermum ammi* (12) and *Cuminum cyminum* (13,14,15) EOs and also on the antibacterial activity of *Artemisia absinthium* (16), *Zataria multiflora Boiss.* (17,18,19), *Pulicaria gnaphalodes* (20), *Trachyspermum ammi* (21) and *Cuminum cyminum* (15) EOs on some food-borne and pathogenic microorganisms. In spite of the importance and long-term use of *Trachyspermum ammi*, *Pulicaria gnaphalodes* and *Artemisia absinthium*, no work has been carried out on the antimicrobial activity of these EOs on pathogenic and non-pathogenic *V. parahaemolyticus*.

The aim of the present study was to determine the chemical composition of some of the most important essential oils of plants cultivated all around Iran which are *Zataria multiflora Boiss., Trachyspermum ammi, Artemisia absinthium, Pulicaria gnaphalodes* and *Cuminum cyminum* and to evaluate their antimicrobial activity against pathogenic and non-pathogenic *V. parahaemolyticus*. Data obtained in this study could help to identify potential essential oils to be applied as food preservatives especially in seafood.

Materials and Methods

Plant material: *Artemisia absinthium, Zataria multiflora Boiss., Pulicaria gnaphalodes, Trachyspermum ammi* and *Cuminum cyminum* plants were used in this study. List of the plants and their properties are given in table 1.

Extraction of Essential Oil (EO): Essential oils were extracted from different parts of the plants (table 1) by hydrodistillation method using a Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulfate and stored in a sterilized vial at 4°C until use.

Table 1. List of the tested essential oils and their properties

Botanical name (family)	Details of plant oils			
	Common name	Origin	Distilled part	Properties
Zataria multiflora Boiss. (Laminaceae)	Avishan-e-shirazi	Shiraz province	Aerial parts	Antiseptic,carminative,stimulant,diaphoretic,diretic,anesthetic, anthelmintic, antidiarrheal and analgesic (45)
Trachyspermum ammi (Apiaceae)	Ajwain	Isfahan province	Seeds	Insecticidal,antispasmodic,stimulant,carminative,antiviral,anti- inflammatory,antipyretic, antifilarial,analgesic,anti-nociceptive and antioxidant(37,21)
Artemisia absinthium (Asteraceae)	Worm wood	Golestane province	Aerial parts	Antiseptic, insect repellent, anthelmintic antimalarial, antiviral,antitumor,spasmolytic, antipyretic, analgesic, antidiabetic (44)
Pulicaria gnaphalodes (asteraceae)	Nufaij	Khorasan province	Aerial parts	antidiarrheal and anti-inflammatory (20)
Cuminum cyminum (Umbelliferae)	Cumin	Kerman province	Seeds	stimulant, carminative, astringent, and as remedy against indigestion, flatulence, diarrhea and toothache(13)

Gas chromatography mass spectrometry (GC/MS) analysis: The EO was analyzed by gas chromatography (GC) (Thermoquest 2000, Manchester, UK). The chromatograph was equipped with Hp5 capillary column (30 × 0.25 mmID× 0.25 µm film thickness) and the data were acquired under the following conditions: initial temperature 50°C, program rate 2.5°C, final temperature 265°C and injector temperature 250°C. The carrier gas was helium at the flow rate of 1.1 ml/min and the split ratio was 1:100. The essential oil was also analyzed by GC mass spectrometry (GC/MS) on the same gas chromatograph coupled with MSD5973. The MS was run in the electron ionization mode, using ionization energy of 70 eV and interface temperature was 250°C. The components of essential oils were identified tentatively by comparing their retention indices and mass spectra with those of Wiley 275 Registry of Mass Spectral Data (22) and literature citations (23,24) and/or by conjunction with authentic samples.

Strain and preparation of inoculums: Lyophilized culture of *V. parahaemolyticus* American Type Culture Collection 43996 and 17802 obtained from Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, was used in this study. Stock culture of the bacteria were transferred to tubes containing 10 ml of brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) and were incubated at 37°C for 24h. The same procedure was repeated one more time. For short time preservation, a loop of the bacteria from the second culture was inoculated on slant BHI agar and was incubated under the same condition and kept at 4°C. A loop of bacteria from the second culture was grown on BHI broth and incubated at 37°C for 6h and then was used to prepare inoculums; the bacterial suspension was adjusted to an optical density of 0.1 at a wave length of 600 nm using a Spectronic 20 spectrophotometer (Milton Roy Company, Rochester, NY) and enumerated by duplicate plating from 10-fold serial dilutions on BHI agar (Merck, Darmstadt, Germany).

Disc diffusion assay: The detection of inhibitory effect of the essential oils on the tested bacteria was carried out by agar disc-diffusion method based on the document M2-A8 of CLSI (2015a) with some modifications (25). The essential oils were tested against *V. parahaemolyticus* ATCC 43996 and 17802. BHI agar medium was prepared containing 5% dimethyl sulfoxide (DMSO) to enhance oil solubility. A suspension of the tested microorganism (0.1 dilution of OD=0.1 at a wave length of 600 nm) was spread on the solid media plates. The plates were kept in fridge before the discs were prepared. Sterile paper discs (6 mm in diameter) were impregnated with10 µl of the oil and placed on the inoculated plates which were then incubated at 37°C for 24h. The diameter of the zones of growth inhibition around each of the discs including the discs diameter was taken as measure of the antimicrobial activity. Each experiment was carried out in duplicate and the mean diameter of the inhibition zone was recorded.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration of the EOs against *V. parahaemolyticus* ATCC 43996 and 17802 was determined using the microdilution broth method based on the document M7-A6 of CLSI (2015b) (26). Sterile 96-well microplates were used for the assay. Stock solutions of the EOs were prepared in 10% (v/v) DMSO. Dilution series of EOs, using BHI broth, were prepared from 0.025 to 0.3% (v/v). Of each dilution, 200 µl were transferred into 96-well microtitre plates, followed by adding 20 µl of respected standardized microorganism suspensions containing 10^4 colony forming units per ml (cfu/ml). A well, consisting of BHI broth, 10% (v/v) DMSO and microorganisms was the growth control. After incubation at 37°C for 24h, the lowest concentrations without visible growth were defined as concentrations that completely inhibited bacterial growth (MICs).

Determination of Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentrations of EOs were determined according to the MIC values, based on Celiktas *et al.* (2007) (27). Of each well showing complete absence of growth, 10 µl were transferred to agar plates (BHI) and incubated at 37°C for 24h. The lowest concentrations of EOs where no viable bacteria were identified were the MBC.

Statistical analyses: Data from disk diffusion assay, MIC and MBC of each EO on both *V. parahaemolyticus* were subjected to Mann-Whitney U Test using SPSS version 22 statistical package. For comparison of the effect of 5 EOs on each *V. parahaemolyticus*, the Kruskal-Wallis test was performed. For all analyses, $P< 0.05$ was considered as statistically significant.

Results

Chemical composition of the EOs: The main constituents of the studied essential oils are presented in table 2. The analysis of the components by gas chromatography mass spectrometry allowed the identification of 76 compounds, accounting for 97.43, 96.96, 96.4, 99.99 and 95.69% of the composition of volatile substances of *Artemisia absinthium*, *Zataria multiflora* Boiss., *Pulicaria gnaphalodes*, *Trachyspermum ammi* and *Cuminum cyminum*, respectively. The major phenolic components of *Z. multiflora* Boiss. EO were carvacrol (73.64%) and thymol (4.31%). The main components of *Trachyspermum ammi* EO were thymol (63.42%), p-cymene (19.01%) and γ-terpinene (16.89%). α-pinene (32.2%) and 1,8-cineole (10.9%) were the major constituents of *P. gnaphalodes* EO. The components of *C. cyminum* EO were cuminaldehyde (29.02%), α-terpinene-7-al (20.70%) and γ-terpinene (12.94%). The main components of *A. absinthium* were β-thujone (29.75%), phellandrene (20.10%) and sabinene (15.81%).

Table 2. Chemical composition (%) of identified compounds in the tested essential oils determined by gas chromatography-mass spectrometry

No.	component	Quantity (%)					RI[a]
		Artemisia absinthium	*Zataria multiflora Boiss.*	*Pulicaria gnaphalodes*	*Trachyspermum ammi*	*Cuminum cyminum*	
1	α-pinene	0.42	0.63	32.2	0.06	0.68	857
2	Sabinene	15.81			0.44		899
3	β-pinene	3.54		0.7		7.72	907
4	Phellandrene	20.10					937
5	α-terpinene	0.52		1.4			942
6	Cymene	7.37					951
7	Delta-3-carene	0.45					953
8	γ-terpinene	1.19	2.27	1.4	16.89	12.94	993
9	α-terpinolele	0.25					1004
10	2,5-octadiene	0.49					1011
11	β-thujone	29.75					1038
12	3-methyl-3-butenyl 3-methyl-2-butenyl ether	0.58					1052
13	4,4,7,7-tetramethyl-delta-octalin-1,6-dione	0.65					1055
14	4-terpineol	3.27		6.5			1088
15	3,7,7-trimethyl-bicyclo(4.1.0)hept-2-ene	0.27					1095
16	(1-methyl-1,2-propadienyl)-cyclopropane	0.65					1106
17	α-copaene	0.38					1293
18	α-cedrene	0.24					1318
19	Trans-caryophyllene	0.34	2.93				1323
20	Delta-selinene	0.32					1341
21	Germacrene D	2.19					1366
22	Alloaromadendrene	0.72		0.2			1369
23	Spiro (tricycle [6.2.1.0 (2,7)] undeca-2,4,6-triene)-7,1′-cyclopropane	6.54					1386
24	Naphthalene,1,2,- dihydro-2,5,8-trimethyl	0.31					1387
25	α-amorphene	0.44					1390
26	Delta-cadinene	0.38		0.6			1393
27	Cis-alpha-bisabolene	0.26					1410
28	1-octen-3-ol		0.29				982
29	3-octanone		0.75				988
30	β-myrcene		0.56				992
31	3-octanol		0.34				996
32	1-phellanderene		0.27	0.9			1003
33	α-tripenene		0.76				1017
34	β-phellanderene		0.33			0.84	1031
35	1,8-cineole		0.52	10.9			1033
36	α-tripinolene		0.26				1088
37	Linalool		1.28	0.9			1101
38	Thymyl methyl ether		0.62				1236
39	Carvacrolmethyl ether		3.15				1248
40	Thymol		4.31	2.7	63.42		1235
41	Carvacrol		73.64				1320
42	Isopiperitone		0.59				1365
43	Carvacryl acetate		1.7				1381
44	α-humulene		0.35				1460
45	Spathulenol		0.58				1587
46	Caryophellene oxide		0.83				1595
47	β-citronellol			8.9			1227
48	α-terpineol			6.9			1191
49	Mertenol			5.6			1194

Table 2. (Continued)

No.	component	Quantity (%)					RI[a]
		Artemisia absinthium	*Zataria multiflora Boiss.*	*Pulicaria gnaphalodes*	*Trachyspermum ammi*	*Cuminum cyminum*	
50	Chrysanthenone			3.7			1123
51	Cadalene			2.2			1662
52	p-cymene			1.7	19.01	8.55	956
53	Filifolene			0.8			1088
54	γ-cadinene			0.8			1523
55	1-terpineol			0.7			1141
56	Neral			0.7			1239
57	Trans-sabinene-hydrate			0.6			1058
58	Cis-chrysanthenol			0.6			1157
59	β-ionone			0.6			1461
60	ledol			0.6			1598
61	α-terpinolene			0.5			1087
62	Nerol oxide			0.5			1153
63	Cis-calamenene			0.5			1516
64	α-thujene			0.4	0.07		852
65	Camphor			0.4			1145
66	α-citral			0.4			1270
67	Eugenol methyl ether			0.4			1376
68	Caryophyllene epoxide			0.3			1593
69	α-murolene			0.2			1499
70	β-ocimene X				0.1		958
71	γ-Terpinene-7-al					8.90	1304
72	α-terpinene-7-al					20.70	1289
73	Cuminaldehyde					29.02	1247
74	Dihydrocarvone (cis)					4.45	1195
75	Myrcene					1.1	991
76	α-phellandrene					0.79	1003
	TOTAL	**97.43**	**96.96**	**96.4**	**99.99**	**95.69**	

[a]RI: Retention Index

Antimicrobial activity of the tested EOs: Table 3 shows the antimicrobial activity of the essential oils as determined by the agar disc diffusion test. Of the five tested EOs, four oils exhibited an antimicrobial effect against both strains of *V. parahaemolyticus*, but *A. absinthium* did not show any antimicrobial activity. In all EOs tested, pathogenic *V. parahaemolyticus* ATCC 43996 showed more sensitivity than non-pathogenic *V. parahaemolyticus* ATCC 17802. The strongest EO against pathogenic *V. parahaemolyticus* were *T. ammi* and *C. cyminum*, equally (31 mm inhibition zone). Non- pathogenic *V. parahaemolyticus* showed the most sensitivity against *Z. multiflora* Boiss (27 mm inhibition zone). MIC and MBC values of these essential oils against *V. parahaemolyticus* are shown in table 4. MIC values ranged between 0.025 and >0.3% (v/v), and MBC values, between 0.05 and >0.3% (v/v). The results of MIC and MBC values are relatively in accordance with agar disc diffusion assay. The highest MIC and MBC values against both pathogenic and non-pathogenic *V. parahaemolyticus* belong to *A. absinthium* which showed no antimicrobial activity in disc diffusion assay while the strong antibacterial activity of *Z. multiflora* Boiss. EO was confirmed by the lowest MIC (0.025% against *V. parahaemolyticus* ATCC 17802 and 0.05% against *V. parahaemolyticus* ATCC 43996) and MBC (0.05% against both *V. parahaemolyticus* strains).

Table 3. Antimicrobial activity of the evaluated essential oils against *Vibrio parahaemolyticus*, as detected by the agar disc-diffusion test (mm)

EOs	microorganisms		P-value
	V.parahaemolyticus ATCC 43996	*V.parahaemolyticus* ATCC 17802	
Artemisia absinthium	8(-)[a,b]	6(-)	0.33
Zataria multiflora Boiss.	28±1.41(+++)	27±1.41(+++)	1.00
Pulicaria gnaphalodes	23±2.47(+++)	15(++)	0.33
Trachyspermum ammi	31±1.41(+++)	24±2.82(+++)	0.33
Cuminum cyminum	31±1.41(+++)	21±0.35(+++)	0.33
P-value	0.77	0.69	

[a]Inhibition area including 6 mm disc diameter, expressed as the mean of two replicates ± SD.
[b]Inhibition was scored as: Not sensitive (-) for total diameter smaller than 8 mm, Sensitive (+) for total diameter 9–14 mm, Very sensitive (++) for total diameter 15–19 mm, Extremely sensitive (+++) for total diameter larger than 20 mm according to Ponce *et al.* 2003 (46).

Table 4. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (% v/v) of the studied essential oils against *Vibrio parahaemolyticus* determined by microdilution method.

EOs	MIC % (v/v)		P-value	MBC % (v/v)		P-value
	V.parahaemolyticus ATCC 43996	*V.parahaemolyticus* ATCC 17802		*V.parahaemolyticus* ATCC 43996	*V.parahaemolyticus* ATCC 17802	
Artemisia absinthium	>0.3	>0.3	1.000	>0.3	>0.3	1.00
Zataria multiflora Boiss.	0.05	0.025	0.333	0.05	0.05	1.00
Pulicaria gnaphalodes	0.2	0.2	1.000	0.3	0.3	1.00
Trachyspermum ammi	0.05	0.05	1.000	0.1	0.05	0.33
Cuminum cyminum	0.2	0.2	0.333	0.2	0.3	0.33
P-value	0.61	0.61		0.61	0.61	

Discussion

Zataria multiflora is a thyme-like plant that grows wild only in Iran, Pakistan and Afghanistan (28). To date, a large number of studies have focused on *Z. multiflora* EO: some reported carvacrol as the main compound (29,30), but others reported thymol, the isomer of carvacrol, as the main compound (31). In a research conducted by Basti *et al*. (2007), carvacrol was one of the major phenolic components of *Z. multiflora* EO without any thymol identified (32). In the present study, the major phenolic components of *Z. multiflora* EO obtained from Iran were carvacrol (73.64%) and thymol (4.31%). Similar to our results, Gupta *et al.* (1972) reported that the essential oil of the Indian ecotype consists of 69% phenols containing mainly carvacrol (10). It is clear that geographical variation, cultivar differences, stage of plant growth, preparation process and other factors may influence oil composition both quantitatively and qualitatively (28). Acidic nature of the hydroxyl group in thymol and carvacrol and the involvement of the hydroxyl group in the formation of hydrogen bonds may explain the highest antimicrobial activity (31). Investigations showed that *Z. multiflora* EO can inhibit food-borne pathogenic microorganisms such as *Staphylococcus aureus* (33), *Bacillus cereus* (34), *Sallmonella typhimurium* (17), *Listeria monocytogenes* (18) and *Aspergillus flavus* (19). Mansour *et al.* (2010) showed that *S. aureus* was more sensitive than other tested bacteria (MIC 0.039 % (w/v)) and *Streptococcus pyogenes* and *Proteus mirabilis* (MIC 0.156 % (w/v)) were the most resistant bacteria (35). In the present study, *Z. multiflora* EO had the strongest antibacterial effect on *V. parahaemolyticus* ATCC 17802 (27 mm inhibition zone), while it was in the third position after *Trachyspermum ammi* and *Cuminum cyminum* regarding *V. parahaemolyticus* ATCC 43996 (28 mm inhibition zone). *Z. multiflora* EO had the lowest MIC and MBC values which was 0.05% against *V. parahaemolyticus* ATCC 43996 and 0.025% and 0.05% against *V. parahaemolyticus* ATCC 17802, respectively. In a study conducted by khanjari *et al.* (2013) the growth of pathogenic *V. parahaemolyticus* was significantly affected by *Z. multiflora* EO (36).

Trachyspermum ammi is known as a popular aromatic herb and spice which is usually used in subcontinent, Middle East Asia and some parts of America (37). Mohagheghzadeh *et al.* (2007) showed that *T. ammi* has two chemotypes, thymol and carvacrol (38). Therefore, the *T. ammi* oil tested in this study belonged to the thymol chemotype. In a research conducted by Oroojalian *et al.* (1999) the main constituents of *T. ammi* EO were thymol (48.4%), p-cymene (21.8%), γ-terpinene (21.3%) and β-pinene (2.6%) (12). The main components of the EO in the present study were thymol (63.42%), p-cymene (19.01%), and γ-terpinene (16.89%). As can be seen, the results of the two studies are the same but the thymol concentration in our study was higher without any β-pinene identified. These differences in chemical compositions of the oils could be attributed to environmental effects on the plants (12). The lowest MIC values of *T. ammi* essential oil were found to be 0.01% v/v against *E. coli* and MIC value for *K. pneumonia* was 2.5% v/v (21). MIC and MBC values of *T. ammi* EO against *V. parahaemolyticus* ATCC 43996 in this study were 0.05% v/v which shows its bactericidal effect in such a low concentration. The MIC and MBC values of *T. ammi* EO against *V. parahaemolyticus* ATCC 17802 were 0.05% and 0.1% v/v, respectively. Oroojalian *et al.* (2010) showed that *T. ammi* EO was more active than

C. cyminum against all tested pathogenic bacteria (12). These EOs showed equal inhibition zone against *V. parahaemolyticus* ATCC 43996 (31 mm) in our research, but in MIC and MBC values, *T. ammi* had lower values than *C. cyminum*. The higher antibacterial activity of *T. ammi*, possibly relates to the high amount of thymol and γ-terpinene which have been reported to possess antibacterial properties (12).

Pulicaria gnaphalodes is traditionally used as a flavoring agent in food. The main components of *P. gnaphalodes* from Qom area of Iran as reported by Kazemi *et al.* (2013) were completely different from our result (39). There is an interesting similarity between our study and the study conducted by Weyerstahl *et al.* (1999) regarding two main components (α-pinene 32.2% and 1,8-cineole 10.9%) (11). *V. parahaemolyticus* ATCC 43996 was more sensitive (23 mm inhibition zone) to *P. gnaphalodes* in comparison to *V. parahaemolyticus* ATCC 17802 (15 mm inhibition zone). But MIC and MBC values of *P. gnaphalodes* EO against *V. parahaemolyticus* ATCC 43996 and 17802 were the same: 0.2 and 0.3 % v/v, respectively. Gandomi *et al.* (2015) reported the MIC values of *P. gnaphalodes* against *Staphylococcus aureus* and *Bacillus cereus* were 0.1 and 0.025 v/v, respectively (20).

C. cyminum is the second most popular spice in the world after black pepper (13). The components of *C. cyminum* EO in our study were cuminaldehyde (29.02%), α-terpinene-7-al (20.70%) and γ-terpinene (12.94%) which is completely different from Chinese *C. cyminum* EO and relatively similar to Tunisian *C. cyminum* composition (13,14). Hajlaoui *et al.*(2010) reported that inhibition zone of *C. cyminum* EO against *V. parahemolyticus* ATCC 17802 and 43996 were 15 and 13.3 mm, respectively (13). In this research, inhibition zone of *C. cyminum* on both *V. parahaemolyticus* strains were more than (31 and 21 mm for *V. parahaemolyticus* ATCC 43996 and 17802) what they reported. Under equal conditions, the difference in the diameter of zones of inhibition can be attributed to the techniques employed, but here the more remarkable matter is the relative difference between EO's composition which was previously mentioned. On the other hand, Oussalah *et al.* (2006) showed that *C. cyminum* essential oil which was mainly comprised of cuminal, γ- terpinene, and β-pinene, showed a weak antimicrobial activity against *P. putida* showing a MIC value above 0.8% (40).

Artemisia absinthium is an aromatic plant which is used in fragrant compounds (41). Three chemotypes of wormwood oil are thujones rich oil, sabinene acetate rich oil, and epoxyocimenes rich oil (42). The main components of *A. absinthium* in this study were β- thujone (29.75%), phellandrene (20.10%), and sabinene (15.81%). So, it belongs to thujones rich oil. Chiasson *et al.* (2001) reported β-thujone as a main component (32.1%) in wormwood essential oil from Canada (9). The high amounts of thujones are responsible for antibacterial and antifungal activities of Croatian EO (16). Despite the large amount of thujone, *A. absinthium* EO in this study did not show antibacterial activity in disk diffusion assay, MIC or MBC values. This can be attributed to the antagonistic activity of some trace components in *A. absinthium* EO (43). Besides, thujone isomers are responsible for the hallucinogenic and toxic effect of wormwood oil, due to this, thujones rich chemotypes of wormwood are not appreciated. So, the use of thujone in foods and beverages are strictly regulated or prohibited (41).

Conclusion: The present work is the first research on the antimicrobial activities of *T. ammi*, *P. gnaphalodes* and *A. absinthium* EOs on *V. parahaemolyticus*. The results of this study suggest that *Z. multiflora* Boiss. and *T. ammi* have strong antimicrobial activity against both pathogenic and non-pathogenic *V. parahaemolyticus* which food safety risk still has to be considered because of the risk of seafood cross contamination and undercooking. Because *Z. multiflora* Boiss. is a widely-used dietary plant, there is negligible concern regarding any toxic or severe adverse effects following consumption at pharmacologically relevant doses. However, additional studies regarding toxicity and also in food model systems would be needed to justify and further evaluate the potential of these oils as antibacterial agents in food industry especially marine products.

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