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Original Article

Antimicrobial activity and chemical composition of *Thymus* species and *Zataria multiflora* essential oils

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ABSTRACT

Thymus spp. and *Zataria multiflora* essential oils are known in Iran as thyme oils. The chemical composition and antimicrobial activity were compared of different thyme oils from different regions of Iran. The antimicrobial activities were evaluated using disc diffusion and micro-broth dilution assays. The chemical compositions of the essential oils were analyzed. Thymol, carvacrol, *para*-cymene and linalool were the main components of the thyme essential oils. The geographical region can affect the chemical composition of essential oils. *Aspergillus niger* and *Candida albicans* were the most sensitive microorganisms to thyme oils. Based on minimal inhibitory concentration and minimum lethal concentration values, respectively, the best antimicrobial thyme essential oils were *T. vulgaris*-SH (0.27 μL/mL, 0.41 μL/mL), *T. kotschyanus*-SH (0.37 μL/mL, 0.64 μL/mL) and *T. pubescens*-SE (0.26 μL/mL, 0.75 μL/mL). In broth media, three chemotypes of thyme essential oils showed the highest antimicrobial effects: 1) linalool, thymol and carvacrol chemotype; 2) thymol, carvacrol and *para*-cymene; and 3) high amounts of carvacrol and *para*-cymene. Identification of the role of each component in the antimicrobial effect was not possible and additional software is required to draw the matrices of these reactions.

Introduction

Thymus L. (Labiatae family) has 14 perennial species in Iran, though regardless of the species, they are identified as thyme (Mozaffarian, 1996). However, the genus *Zataria* Boiss (Labiatae family) has one species namely *Z. multiflora* that grows in southern Iran and is called thyme (Mozaffarian, 1996). All *Thymus* spp. (Stahl-Biskup, 1991) and *Z. multiflora* (Mahboubi, 2013) have an aromatic essential oil as a secondary metabolite with the main components being thymol, carvacrol and *para*-cymene. The overall biological activities of thyme essential oils without considering the main components are the same and sometimes, it is possible to substitute one species for another with regard to their antimicrobial effects. Thyme essential oils have different biological activities such as anti-inflammatory (Vigo et al., 2004), analgesic, anti-pyretic (Qadir et al., 2016) and immune-stimulation effects (Soltani et al., 2010). One interesting biological effect of thyme essential oils is their antimicrobial effects which are related to their chemical composition and the geographical location of plants affects the constituents of their essential oils and the potency of their antimicrobial activities (Nzeako et al., 2006). Thyme essential oils with a higher concentration of phenol exhibit greater antimicrobial effects (Burt, 2004; Boskovica et al., 2015). Although, many different studies have evaluated the chemical composition of thyme essential oils and also the related antimicrobial effects (Asllani and Toska, 2003; Boruga et al., 2014; Saei-Dehkordi et al., 2010), up to now, there has not been a study that has compared the chemical compositions and antimicrobial effects of these essential oils.

This study analyzed the chemical compositions of thyme essential oils from different species collected in different locations. Due to the relationship between the chemical composition of the essential oil and antimicrobial effects, the antimicrobial activities of each oil against different kinds of microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*) were evaluated. The aim was to find a relationship between the chemical compositions of essential oils with their antimicrobial activities and to evaluate the effects of geographical region on the chemical compositions of the essential oils.

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Material and methods

Plant materials, essential oil extraction, and chemical analysis

Thirteen samples of aerial parts from different species of thyme (*T. vulgaris* [n = 3], *T. daenensis* [n = 2], *T. kotschyanus* [n = 3], *T. fedtschenkoi* [n = 1], *T. pubescens* [n = 1] and *Z. multiflora* [n = 3]) at the full flowering stage were collected from different parts of Iran. The herbarium samples were identified and authenticated (Table 1). The samples were dried, cut into small pieces and subjected to hydro-distillation for 3 h. The essential oils were dried and kept until analysis.

The chemical compositions of essential oils were identified using gas chromatography (GC) and GC-mass spectrometry (GC-MS). The GC and GC-MS analyses were conducted on a Trace mass spectrometer (Thermo Quest-Finnigan; Thermo Electron Corporation; Dallas, TX, USA) with a capillary column of DB-5 (30 m × 0.25 mm, film thickness 0.25 μm) and coupled with a 5973 network mass selective detector system, respectively. The oven temperature program was initiated at 60 °C, held for 1 min then raised to 250 °C at a rate of 3 °C/min and held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min with a split ratio equal to 1/100 injector. The detector and injector temperatures were at 250 °C and 230 °C, respectively. Components of essential oils were identified by comparison with retention indices relative to homologous series of n-alkanes and using libraries of Wiley 275.L and Wiley 7n.1, as well as a comparison of the fragmentation pattern of the mass spectra with data published in the literature (Adams, 2001).

Microbial strain and antimicrobial evaluations

S. aureus ATCC 25923, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404 were the microorganisms tested using antimicrobial screenings. The microbial strains were cultured on Sabauraud dextrose agar for fungi and Muller Hinton agar for bacteria and were incubated under aerobic conditions at 35 ± 2 °C for 24 h and at 23 ± 2 °C for 48 h for bacteria and fungi, respectively. One or two colonies of each microorganism were suspended in normal saline and the turbidity was adjusted to 0.5 McFarland (1×10^8 colony forming units (CFU)/mL and 1×10^6 CFU/mL for bacteria and fungi, respectively).

Disc diffusion and micro-broth dilution assays were used to evaluate the antimicrobial activities of the essential oils. In the disc diffusion method, the prepared microbial suspensions were cultured on suitable solid media (Sabauraud dextrose agar for fungi and Muller Hinton agar for bacteria) using sterilized cotton swabs. The blank discs impregnated with 0.75 and 1 μL essential oil were put on cultured media and were incubated under suitable conditions (aerobically at 35 ± 2 °C for 24 h and at 23 ± 2 °C for 48 h for bacteria and fungi, respectively). Antibiotics (gentamicin 10 μg/disc, vancomycin 30 μg/disc and amphotericin B 10 μg/disc) were used as controls. The diameters of inhibition zones (in millimeters) were determined. Each concentration was repeated three times and the results were analyzed statistically (Clinical and Laboratory Standards Institute, 2009).

The prepared adjusted microbial suspensions were diluted to 1×10^6 CFU/mL and 1×10^4 CFU/mL for bacteria and fungi, respectively. Then, 100 μL of serially two-fold diluted essential oil in media broth (16–0.03 μL/mL), and 100 μL of diluted microbial suspension were added to each well and incubated under suitable conditions. The first well that had no visual turbidity in broth media was defined as the minimal inhibitory concentration (MIC) in μl/mL and the first well that had no growth on suitable media was defined as the minimal lethal concentration (MLC), both expressed in microliters per milliliter (Clinical and Laboratory Standards Institute, 2008).

Statistical analysis

The SPSS for Windows (IMB, SPSS22, Inc.; Chicago, IL, USA) software was used for data and statistical analysis. Numerical data were analyzed using one-way analysis of variance to compare the mean values. A probability equal to or less than 5% was considered statistically significant.

Results and discussion

Plant materials, essential oil yields, and chemical composition

Three samples of *T. vulgaris* were collected from different parts of Iran (Yazd, Shahrood and Kashan). The essential oil yields were 2–2.3%. *T. vulgaris* essential oil (yield: 2.1% weight per weight; w/w) from Kashan (Central Iran) had a high thymol content (74.5%), followed by *para*-cymene (7.5%), γ-terpinene (5.5%) and carvacrol (3.7%). *T. vulgaris* essential oil from Shahrood (yield: 2.3% w/w) had 62.5% carvacrol as the main component, followed by *para*-cymene (9%), α-copaene (6.0%) and isopinocamphene (3.6%). The amount of thymol was about 0.5% in this sample. Thymol (43.3%), carvacrol (11.5%), *para*-cymene (11.4%), γ-terpinene (4.7%) and borneol (3.9%) were the main components of *T. vulgaris* essential oil from Yazd (yield: 2.0% w/w).

The yields of essential oil from the two samples of *T. daenensis* were 2–2.2%. Thymol (59.4%, 17.7%), carvacrol (7.12, 50.4%), *trans*-caryophyllene (5.34, 1.1%), borneol (3.94, 3.27%), *para*-cymene (4.11, 2.6%), γ-terpinene (3.96, 3.5%) and thymol methyl ether (0, 4.41%) were the main components of *T. daenensis* essential oils from Kashan and Shahrood, respectively. The yields of essential oils were 2.0% and 2.2%, respectively.

The yields of essential oils from three samples of *T. kotschyanus* were in the range 0.68–1.1%. The lowest amount of essential oil yield was for *T. kotschyanus* from Semnan (0.68%).

Thymol (39.8%), geraniol (14.4%) and 1,8-cineol (5.2%) were the main components of *T. kotschyanus* essential oil from Semnan, followed by borneol (4.5%), geranyl acetate (4.1%), *para*-cymene (3.8%) and carvacrol (2.7%), while linalool (31.1%), carvacrol (14.3%), thymol (13.1%) and *trans*-carvyl acetate (10.9%) were the main components of *T. kotschyanus* essential oil from Yazd (yield: 1.0% w/w). *T. kotschyanus* essential oil from Shahrood had thymol (61.2%), carvacrol (5.4%), *para*-cymene (5.3%) and linalool (4.6%) as the main components (yield: 1.1% w/w).

The essential oil yield for *T. fedtschenkoi* and *T. pubescens* was 0.8% and 0.68% (w/w), respectively. Thymol (50.6%), *para*-cymene (7.7%), carvacrol (6.6%), borneol (4.1%) were the main components of *T. fedtschenkoi* essential oil. Thymol (26.6%), carvacrol (27.0%), linalool (8.4%), *para*-cymene (8.2%), 1,8-cineol (4.3%) and borneol (3.6%) were the main components of *T. pubescens* essential oil.

Extraction of the essential oils from the three samples of Iranian thyme (*Z. multiflora*) produced yields in the range 1.8–2.8% w/w. Thymol (25.8–41.2%), carvacrol (1.5–34.3%), carvacrol methyl ether (1.8–28.3%), *para*-cymene (2.3–12.1%), linalool (1.3–6.5%), γ-terpinene (0.92–6.5%) and α-terpinene (0.26–5.1%) were the main components of *Z. multiflora* essential oil. *Z. multiflora* essential oil from Fars and Shahrood had higher levels of thymol (41.2%) and carvacrol (34.3%), respectively (Fig. 1, Table 1).

Many studies have evaluated the chemical composition of essential oils and have noted that the chemical composition of essential oils was affected by harvesting time, the collection location, environmental conditions, drying method, essential oil extraction method, genetic diversity and the growth stage (Nouri et al., 2005; Zarshenas and Krenn, 2015).

Oxygenated monoterpenes are the most known components in *T. daenensis* essential oil and the amount of thymol has been

Table 1
Chemical composition of essential oils using gas chromatography (GC) and GC-mass spectrometry.

Compound	RI	TVK	TVSH	TVY	TDK	TDSH	TKSE	TKY	TKSH	TF	TP	ZMSH	ZMJ	ZMF
α-Thujene	924	–	0.41	0.09	–	0.59	0.37	0.13	0.49	1.30	0.50	0.39	0.90	–
α-Pinene	932	0.67	0.45	0.76	1.16	0.31	0.61	1.05	0.74	1.31	0.60	3.13	4.36	0.02
Camphene	947	0.42	0.42	0.90	1.05	0.28	0.70	0.99	0.69	1.50	0.55	0.19	0.24	0.03
Sabinene	972	0.06	0.05	–	–	0.07	–	–	–	–	–	–	–	–
β-Pinene	975	0.14	0.24	–	0.39	0.21	0.17	–	0.21	1.00	0.72	0.68	1.16	0.29
1-Octen-3-ol	976	–	–	1.97	–	–	–	0.81	–	–	–	–	–	–
3-Octanone	981	–	–	0.11	0.13	1.31	0.15	0.11	–	0.07	0.48	0.60	–	0.52
β-Myrcene	987	0.56	0.22	0.48	1.59	0.39	0.17	2.01	0.90	0.91	0.33	0.98	0.10	0.07
3-Octanol	993	–	0.02	0.16	–	1.08	0.13	0.53	–	0.20	0.15	0.24	1.72	0.31
α-Phellandrene	1003	0.40	0.07	0.07	0.26	0.10	–	–	0.17	0.14	–	0.14	0.35	0.03
δ-3-Carene	1009	–	0.05	–	0.09	–	–	–	0.07	0.08	–	0.04	0.07	–
α-Terpinene	1015	–	0.51	0.96	1.35	0.9	0.11	0.18	1.00	1.15	0.51	1.57	5.10	0.26
p-Cymene	1023	7.45	9.02	11.40	4.11	2.56	3.80	4.01	5.31	7.69	8.23	5.67	12.10	2.27
Limonene	1027	–	0.31	0.35	0.45	–	0.31	–	0.45	0.66	0.28	0.60	0.50	0.17
1,8-Cineole	1029	0.36	0.89	2.57	1.45	1.74	5.20	0.55	0.38	1.71	4.26	0.95	0.03	0.68
β-cis-Ocimene	1034	–	–	–	–	–	–	–	–	–	0.08	0.03	0.10	0.04
γ-Terpinene	1056.3	5.54	0.44	4.69	3.96	3.50	0.09	1.23	1.93	3.16	1.41	4.59	6.50	0.92
cis-Sabinene hydrate	1065	0.40	0.44	0.75	0.32	1.67	0.26	0.22	0.60	2.83	1.49	0.09	–	–
cis-Linalool oxide	1070	0.20	–	–	–	–	–	–	–	–	–	–	–	0.15
α-Terpinolene	1087	0.31	–	–	0.14	0.10	–	–	0.12	0.08	–	0.18	0.2	0.32
Linalool	1097	–	0.04	2.92	0.83	2.00	0.45	31.07	4.60	2.76	8.42	3.12	1.3	6.52
Nonanal	1102	–	–	–	–	–	0.16	–	–	–	–	–	–	–
α-Thujone	1105	–	1.13	–	–	0.14	–	–	0.06	–	–	–	–	–
β-Thujone	1116	–	0.13	–	–	0.04	–	–	–	–	–	–	–	–
Pinovarveol	1142	–	–	–	–	–	0.13	–	–	0.07	–	–	–	–
(+)-2-Bornanone	1146	–	–	–	–	–	–	–	–	–	0.42	–	–	–
Camphor	1146	–	0.04	0.19	–	0.52	0.16	–	–	0.70	0.33	–	–	–
Borneol	1169	–	0.04	5.85	3.94	3.27	4.47	5.69	3.84	4.13	3.55	0.33	–	0.35
Isopinocamphone	1177	–	3.61	–	–	–	–	–	–	–	–	–	–	0.09
Terpinen-4-ol	1180	–	0.07	1.47	1.22	1.29	0.53	0.67	0.89	0.90	0.98	1.41	1.96	2.25
cis-3-Hexenyl butyrate	1186	–	–	–	–	–	–	–	0.14	–	–	–	–	–
p-Cymen-8-ol	1191	–	0.75	–	–	–	0.10	0.13	–	–	–	–	–	–
cis-Dihydrocarvone	1200	–	–	–	–	0.20	0.13	0.16	0.10	–	–	–	–	–
γ-Terpineol	1201	–	–	–	–	–	–	–	–	–	–	0.72	–	0.46
α-Terpineol	1203	–	0.06	0.59	0.27	0.25	0.26	0.23	0.09	0.10	0.50	0.86	–	3.69
p-Allylanisole	1204	–	–	–	–	–	–	–	–	0.12	–	–	–	–
trans-Dihydrocarvone	1210	–	0.16	–	0.10	–	–	–	–	0.21	–	–	–	–
Bornyl formate	1229	–	0.11	0.10	–	–	–	–	–	–	–	–	–	–
Nerol	1232	–	–	–	–	–	0.67	–	–	–	–	–	–	–
cis-Carveol	1233	–	–	–	–	–	–	1.24	–	–	–	–	–	–
Thymol methyl ether	1237	–	0.06	0.09	–	4.41	2.50	0.25	0.16	–	1.22	1.12	–	0.20
Neral	1241	–	–	–	–	–	0.39	–	–	–	–	–	–	–
Carvacrol methyl ether	1244	–	0.05	0.18	2.05	–	0.32	0.37	0.75	–	1.49	1.88	2.30	28.32
Geraniol	1256	–	–	0.3	–	–	14.43	–	–	–	–	–	–	–
Geranial	1269	–	–	–	–	–	0.63	–	–	–	–	–	–	–
Thymoquinone	1271	–	1.16	–	–	0.20	0.23	–	0.21	0.32	0.18	–	–	–
Bornyl acetate	1283	–	–	–	–	–	0.55	–	–	–	–	–	–	–
Anethole	1286	–	1.07	–	–	–	–	–	0.21	–	0.08	–	–	–
p-Cymen-7-ol	1289	–	–	0.82	–	–	–	–	–	–	–	–	–	–
Thymol	1306	74.50	0.49	43.30	59.40	17.70	39.87	13.14	61.20	50.61	26.58	25.80	29.40	41.16
Carvacrol	1311	3.65	62.50	11.50	7.12	50.40	2.64	14.34	5.43	6.58	27.02	34.30	23.90	1.50
cis-Carvyl acetate	1350	–	–	–	–	–	–	10.87	–	–	–	–	–	–
Thymol acetate	1353	–	–	0.09	–	–	–	–	–	–	–	1.30	1.03	0.20
Carvacrol acetate	1371	–	–	–	–	–	–	–	–	–	–	2.26	0.8	2.24
α-Copaene	1374	–	6.02	–	–	–	–	–	–	–	–	–	–	–
Isobornyl propionate	1375	–	–	0.38	–	–	–	–	–	–	–	–	–	–
Geranyl acetate	1380	–	–	–	–	–	4.11	–	–	–	–	–	–	–
(−)-β-Bourbonene	1383	–	0.06	–	–	–	–	–	–	–	0.14	–	–	–
trans-Caryophyllene	1420	1.86	3.48	1.45	5.34	1.10	–	3.54	3.96	3.54	2.90	2.74	–	2.20
Aromandendrene	1438	0.50	0.13	–	0.06	0.10	0.09	–	0.08	–	–	1.20	0.14	0.19
Citronellyl propionate	1440	–	–	0.09	–	–	–	–	–	–	–	–	–	–
Ledene	1494	–	0.17	–	0.15	0.14	–	–	0.12	0.24	–	0.69	0.8	0.97
α-Humulene	1453	0.12	0.11	–	0.2	0.05	0.12	0.23	0.14	0.23	0.08	0.15	0.2	0.07
alloAromadendrene	1459	–	–	–	–	–	0.13	–	–	–	–	0.09	0.12	1
Geranyl propionate	1471	–	–	0.29	–	–	–	–	–	–	–	–	–	–
γ-Muurolene	1475	–	0.16	0.07	–	0.14	–	–	0.13	–	0.1	–	–	–
Germacrene D	1479	–	–	–	–	–	–	0.37	–	–	0.11	–	–	–
Dehydroaromadendrene	1488	–	–	–	–	–	–	–	–	–	–	–	–	0.12
Bicyclogermacrene	1494	–	–	–	–	–	0.09	–	–	–	–	–	–	–
Viridflorene	1496	–	–	–	–	–	–	–	–	–	0.12	–	–	–
Bicyclogermacrene	1497	–	–	–	–	–	–	0.41	–	–	–	–	–	–
β-Bisabolene	1506	0.33	0.53	–	0.13	0.6	0.22	0.27	0.13	0.08	0.12	–	–	–
γ-Cadinene	1512	–	0.11	0.15	–	0.2	–	0.14	0.51	–	0.16	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Compound	RI	TVK	TVSH	TVY	TDK	TDSH	TKSE	TKY	TKSH	TF	TP	ZMSH	ZMJ	ZMF
Myristicine	1521	–	1.57	0.76	–	–	–	0.39	–	–	–	–	–	–
(E)-α-Bisabolene	1543	–	0.28	–	–	0.06	0.49	–	–	–	–	–	–	–
Hedycaryol	1550	–	–	–	–	–	–	0.18	–	–	–	–	–	–
Geranyl butanoate	1556	–	–	–	–	–	0.13	–	–	–	–	–	–	–
δ-Cadinene	1524	–	–	–	–	0.7	–	–	–	0.13	0.22	–	–	–
(E)-α-Bisabolene	1539	0.73	–	–	–	–	–	–	0.31	0.23	–	–	–	–
Elemicin	1555	0.20	0.15	0.12	–	–	–	–	–	–	–	–	–	–
E-Nerolidol	1564	–	–	–	–	–	–	–	–	0.26	–	–	–	–
Thymohydroquinone	1567	–	–	–	–	–	–	–	–	1.26	–	–	–	–
Spathulenol	1578	0.08	0.47	0.17	–	0.22	1.85	0.52	0.11	0.54	0.9	0.9	–	0.93
Caryophyllene oxide	1583	0.34	1.2	2.5	–	0.25	2	1.56	0.69	1.56	2.51	1.03	–	1.27
Neryl isovalerate	1597	–	–	–	–	0.55	0.18	–	–	–	–	–	0.06	0.06
γ-Eudesmol	1623	–	–	–	–	–	–	–	–	0.11	–	–	–	–
τ-Cadinol	1640	–	–	0.39	–	0.39	–	–	1	–	0.65	–	–	–
α-Muurolol	1642	–	–	–	–	–	0.18	0.59	–	0.14	–	–	–	–
α-Cadinol	1658	–	–	0.13	–	–	–	–	1.73	0.09	–	–	–	–
Aromadendrene oxide	1673	–	–	0.24	–	–	–	–	–	–	0.16	–	–	–
Apiol	1682	–	0.58	0.25	–	0.26	–	0.11	0.32	–	–	–	–	0.08
Farnesol	1691	–	–	–	–	–	–	–	–	–	0.21	–	–	–
Shyobunol	1693	–	–	–	–	–	–	1.09	–	–	–	–	–	–
Isobicyclogermacrenal	1733	–	–	–	–	–	0.37	–	–	–	–	–	–	–
Verticiol	2142	–	–	–	–	–	–	–	–	–	0.43	–	–	–
Tridecane	2297	–	–	–	–	–	0.09	–	–	–	–	–	–	–
Pimara-7,15-dien-3-ol	2312	–	–	–	–	–	–	–	–	–	0.83	–	–	–
Tetradecane	2397	–	–	–	–	–	0.27	–	–	–		–	–	–
		98.83	99.98	99.85	97.26	99.99	91.01	99.38	99.99	98.73	100.00	99.97	95.45	100.00

RI = Retention index; TVK = *T. vulgaris-K*; TVY = *T. vulgaris-Y*; TVSH = *T. vulgaris-SH;* TDK = *T. daenensis-K*; TDSH = *T. daenensis-SH;* TDSH = *T. kotschyanus-SE;* TKY = *T. kotschyanus-Y*; TKSH = *T. kotschyanus-SH*; TF = *T. fedtschenkoi-SE*; TP = *T. pubescens-SE*; ZMJ = *Z. multiflora-J*; ZMF = *Z. multiflora-F*; *ZMSH Z. multiflora-SH*.

reported in the range 4.2–85.5% and the amount of thymol was affected by the harvesting time and was high in the flowering stage (Golparvar et al., 2015). Carvacrol was reported as the main component of *T. daenensis* essential oil with geraniol, geranyl acetate, borneol and 1,8-cineole being other reported components of *T. daenensis* essential oil (Nouri et al., 2005).

Seven different chemotypes were reported for *Z. multiflora* essential oil: 1) thymol, carvacrol and linalool; 2) thymol, carvacrol, *para*-cymene; 3) a high amount of thymol and low amounts of carvacrol and *para*-cymene; 4) carvacrol and linalool; 5) linalool and linalyl acetate; 6) carvacrol, γ-terpinene, α-pinene; and 7) thymol, *para*-cymene and borneol (Mahboubi, 2013). Two samples of *Z. multiflora* essential oils had high amounts of thymol and low amounts of carvacrol and *para*-cymene. Terpenoids play an important role in the biological activities of thyme oils. For example, indicator terpenoid compounds in thyme oil such as monoterpenoids and phenolic compounds such as thymol and carvacrol are present in almost all species of *Thymus* sp. and *Zataria multiflora* and hydrocarbon monoterpenes such as *para*-cymene and γ-terpinene are the second group of components in thyme oils (Mahboubi, 2013).

Antimicrobial activities of thyme oils

Among the different screened microorganisms, *P. aeruginosa* was more resistant to thyme essential oil with MIC values in the range 1.8–4.0 μL/mL and MLC values of 3.25–9.0 μL/mL. There was no inhibition zone diameter for *P. aeruginosa* with the thyme essential oils. The lowest MIC and MLC values, respectively, were for *T. vulgaris*-SH (2 μL/mL, 4 μL/mL) and *T. kotschyanus*-SH (1.8 μL/mL, 3.25 μL/mL) against *P. aeruginosa*. *T. vulgaris*-SH and *T. kotschyanus*-SH were containing high amounts of carvacrol (62.5%) and thymol (61.2%), respectively. Resistance has been reported of *P. aeruginosa* to thymol and carvacrol (Sivropoulou et al., 1996).

Regardless of *P. aeruginosa*, among different kind of microorganisms (Gram-positive, negative, molds and yeast), *C. albicans* and *A. niger* were the most sensitive microorganisms to thyme oils and the effects of thyme oils on *A. niger* were more inhibitory than on *C. albicans*. Although the MIC values for *A. niger* were smaller than the MIC values for *C. albicans*, the MLCs were slightly larger than the MICs for *C. albicans*. The sensitivity of *S. aureus* and *E. coli* to thyme essential oils depended on the kinds of thyme oil. *S. aureus* was more sensitive to *T. vulgaris*-Y, *T. kotschyanus*-SH, *T. kotschyanus*-Y and *Z. multiflora*-SH than *E. coli* (Table 2).

Regardless of the microorganism, based on the disc diffusion method (Table 3), *Z. multiflora-F* had the highest inhibition zone diameter (23.1 mm), followed by *T. vulgaris-SH* (19.7 mm), *T. daenensis-K* (19.1 mm), *T. fedtschenkoi-SH* (17.5 mm) and *T. fedtschenkoi-SE* (17.2 mm). Based on the MIC and MLC values, respectively, the best antimicrobial thyme essential oils were *T. vulgaris-SH* (0.27 μL/mL, 0.41 μL/mL), *T. kotschyanus-SH* (0.37 μL/mL, 0.64 μL/mL) and *T. pubescens-SE* (0.26 μL/mL, 0.75 μL/mL). Based on the MIC and MLC values, respectively, *T. kotschyanus-Y* (1.2 μL/mL, 1.7 μL/mL) and *T. daenensis-SH* (1.3 μL/mL, 1.8 μL/mL) had weaker antimicrobial effects than the others, followed by *T. kotschyanus-SE* (0.81, 1.2 μL/mL). Therefore, the matrix of tested materials can affect the antimicrobial effects of the essential oil. In broth media, three chemotypes of thyme essential oils showed the highest antimicrobial effects: 1) linalool, thymol and carvacrol chemotype; 2) thymol, carvacrol and *para*-cymene; and 3) high amounts of carvacrol and *para*-cymene. *Para*-cymene was present in all the tested samples in the range 2.27–12.1%. The highest (12.1%) and lowest amount (2.27%) of *para*-cymene were present in *Z. multiflora* essential oil from Jahroom and Fars, respectively. As the proposed combinations (thymol, carvacrol and *para*-cymene) were responsible for antimicrobial activity of essential oil, it was expected that *T. vulgaris*-Y [(thymol (43.3%), carvacrol (11.5%) and *para*-cymene (11.4%)] and *T. daenensis*-SH [(thymol (17.7%), carvacrol (50.4%) and *para*-cymene (2.6%)] would also show high antimicrobial activity, but in spite of a fair antimicrobial effect, they were weaker than *T. pubescens*-SE [(thymol (26.6%), carvacrol (27.0%) and *para*-cymene (8.2%)]. The reason for this phenomena may have been the synergistic effects of thymol, or carvacrol with

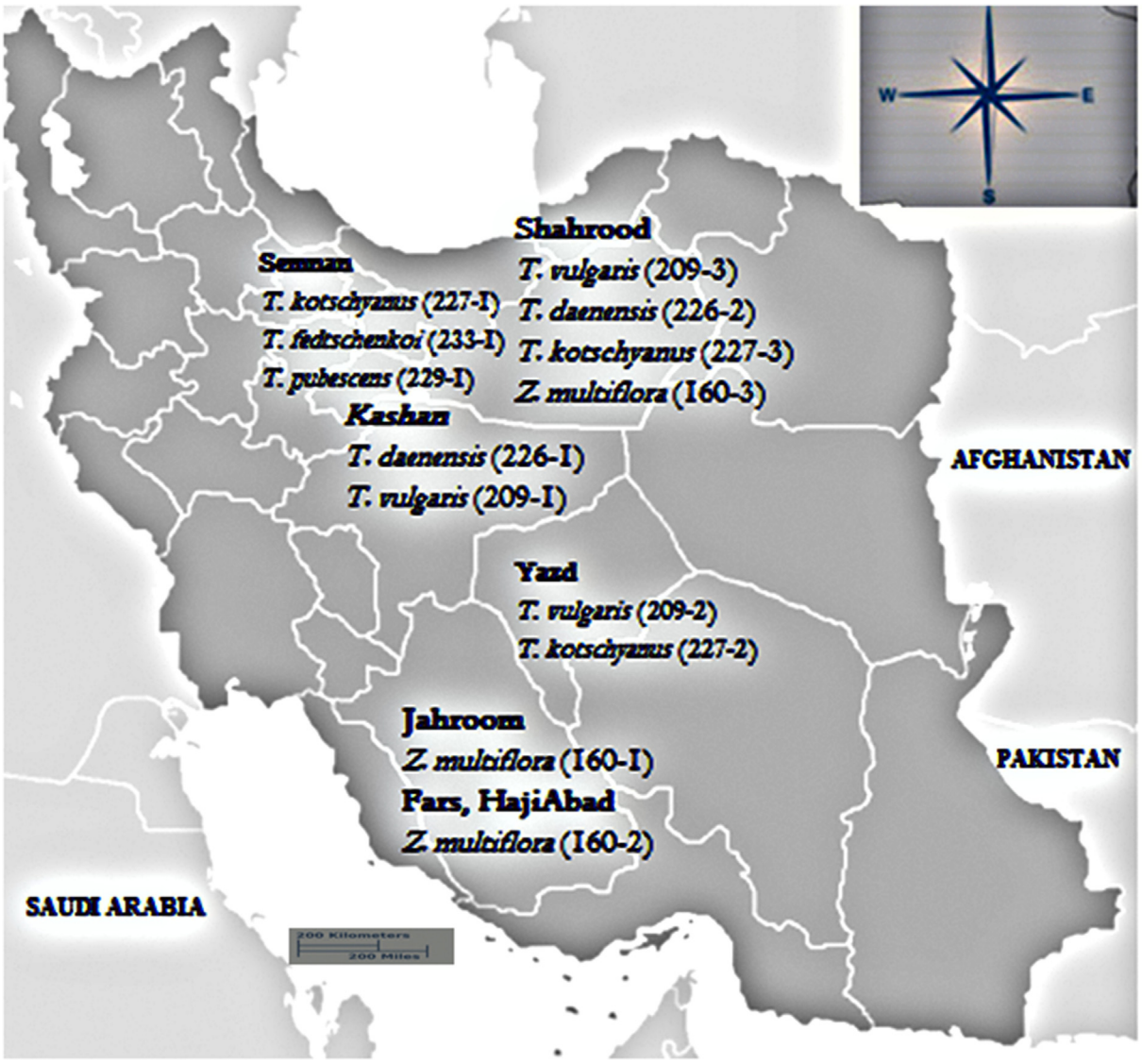

Fig. 1. *Thymus* spp. and *Z. multiflora* from different regions in Iran (herbarium identification in parentheses).

Table 2
Antimicrobial activity of different essential oils against microorganisms (*S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger*).

Essential oil	Microorganism	MIC ± SD (μL/mL)	MBC ± SD (μL/mL)	Inhibition zone ± SD (mm)	
				0.75	1.0
T. vulgaris-B	*S. aureus*	0.41 ± 0.19	0.50 ± 0.67	9.50 ± 1.20	15.30 ± 1.28
	E. coli	0.27 ± 0.16	0.33 ± 0.50	9.20 ± 1.30	9.50 ± 1.20
	C. albicans	0.17 ± 0.18	0.25 ± 0.60	8.10 ± 1.20	10.60 ± 1.20
	A. niger	0.15 ± 0.18	0.40 ± 0.60	6.20 ± 1.20	7.77 ± 1.28
	P. aeruginosa	3.00 ± 0.19	6.00 ± 0.67	-[a]	–
T. vulgaris-SH	*S. aureus*	0.22 ± 0.19	0.22 ± 0.60	34.17 ± 0.80	27.04 ± 0.90
	E. coli	0.21 ± 0.16	0.25 ± 0.55	11.18 ± 1.30	10.34 ± 1.28
	C. albicans	0.12 ± 0.18	0.14 ± 0.60	11.06 ± 1.30	22.13 ± 1.28
	A. niger	0.08 ± 0.28	0.18 ± 0.95	6.20 ± 1.28	11.50 ± 1.28
	P. aeruginosa	2.00 ± 0.39	4.00 ± 1.30	–	–
T. vulgaris-Y	*S. aureus*	0.23 ± 0.15	0.23 ± 0.50	11.22 ± 1.30	15.43 ± 1.28
	E. coli	0.36 ± 0.15	0.39 ± 0.51	11.01 ± 1.30	12.15 ± 1.28
	C. albicans	0.16 ± 0.18	0.30 ± 0.60	8.383 ± 1.30	22.22 ± 1.28
	A. niger	0.06 ± 0.39	0.06 ± 1.40	–	9.10 ± 1.28
	P. aeruginosa	3.50 ± 0.19	5.50 ± 0.67	–	–
T. daenensis-B	*S. aureus*	0.28 ± 0.19	0.31 ± 0.67	17.20 ± 1.20	20.60 ± 1.20
	E. coli	0.15 ± 0.16	0.15 ± 0.50	16.20 ± 1.20	19.80 ± 1.20
	C. albicans	0.08 ± 0.18	0.13 ± 0.60	13.10 ± 1.20	27.20 ± 1.28
	A. niger	0.06 ± 0.18	0.20 ± 0.60	6.20 ± 1.30	31.90 ± 1.20
	P. aeruginosa	3.25 ± 0.19	5.00 ± 0.67	–	–

(*continued on next page*)

Table 2 (*continued*)

Essential oil	Microorganism	MIC ± SD (μL/mL)	MBC ± SD (μL/mL)	Inhibition zone ± SD (mm)	
				0.75	1.0
T. daenensis −SH	*S. aureus*	0.32 ± 0.15	0.46 ± 0.51	16.00 ± 1.28	17.70 ± 1.28
	E. coli	0.25 ± 0.23	0.25 ± 0.80	12.11 ± 1.30	11.82 ± 1.28
	C. albicans	0.12 ± 0.18	0.20 ± 0.60	9.55 ± 1.28	23.79 ± 1.28
	A. niger	0.03 ± 0.39	0.50 ± 1.35	6.20 ± 1.28	11.78 ± 1.28
	P. aeruginosa	3.70 ± 0.15	5.14 ± 0.51	–	–
T. kotschyanus -SE	*S. aureus*	0.50 ± 0.19	0.51 ± 0.67	18.01 ± .90	14.00 ± 0.90
	E. coli	0.23 ± 0.16	0.46 ± 0.55	7.85 ± 1.30	11.00 ± 1.28
	C. albicans	0.15 ± 0.18	0.30 ± 0.60	7.73 ± 1.28	10.90 ± 1.28
	A. niger	0.13 ± 0.19	0.31 ± 0.67	6.20 ± 1.28	6.38 ± 1.28
	P. aeruginosa	3.50 ± 0.19	5.00 ± 0.67	–	–
T. kotschyanus −SH	*S. aureus*	0.18 ± 0.19	0.22 ± 0.67	22.10 ± 1.30	20.90 ± 1.28
	E. coli	0.21 ± 0.13	0.27 ± 0.45	12.70 ± 1.30	15.30 ± 1.28
	C. albicans	0.09 ± 0.18	0.15 ± 0.60	11.50 ± 1.30	26.00 ± 1.40
	A. niger	0.11 ± 0.15	0.214 ± 0.5	6.20 ± 1.28	27.00 ± 1.28
	P. aeruginosa	1.80 ± 0.19	3.25 ± 0.70	–	–
T. kotschyanus -Y	*S. aureus*	0.44 ± 0.19	0.43 ± 0.67	7.23 ± 1.28	8.97 ± 1.28
	E. coli	0.58 ± 0.16	0.92 ± 0.55	7.18 ± 1.28	7.43 ± 1.28
	C. albicans	0.37 ± 0.18	0.40 ± 0.60	6.42 ± 1.40	6.20 ± 1.28
	A. niger	0.23 ± 0.13	0.284 ± 0.4	–	6.20 ± 1.28
	P. aeruginosa	4.00 ± 0.15	5.71 ± 0.51	–	–
T. fedtschenkoi -SE	*S. aureus*	0.28 ± 0.19	0.31 ± 0.67	25.40 ± 0.90	21.98 ± 0.50
	E. coli	0.23 ± 0.16	0.37 ± 0.55	11.10 ± 1.20	13.48 ± 1.20
	C. albicans	0.09 ± 0.18	0.13 ± 0.60	9.90 ± 1.28	24.90 ± 1.20
	A. niger	0.07 ± 0.16	0.18 ± 0.55	8.24 ± 1.00	9.20 ± 1.28
	P. aeruginosa	3.20 ± 0.19	3.50 ± 0.67	–	–
T. pubescens -SE	*S. aureus*	0.250 ± 0.39	0.50 ± 1.35	15.15 ± 0.90	14.94 ± 0.91
	E. coli	0.23 ± 0.16	0.42 ± 0.55	10.00 ± 1.28	9.39 ± 1.28
	C. albicans	0.12 ± 0.18	0.23 ± 0.60	8.71 ± 1.30	19.54 ± 1.28
	A. niger	0.11 ± 0.16	0.35 ± 0.55	6.20 ± 1.28	7.15 ± 1.28
	P. aeruginosa	2.0 ± 0.39	8.00 ± 1.35	–	–
Z. multiflora −SH	*S. aureus*	0.13 ± 0.39	0.12 ± 1.35	15.08 ± 1.20	15.63 ± 1.40
	E. coli	0.25 ± 0.18	0.30 ± 0.60	11.20 ± 1.28	11.04 ± 1.28
	C. albicans	0.12 ± 0.18	0.17 ± 0.60	10.19 ± 1.30	20.12 ± 1.28
	A. niger	0.11 ± 0.13	0.14 ± 0.45	6.20 ± 1.28	10.33 ± 1.28
	P. aeruginosa	2.50 ± 0.19	4.00 ± 0.67	–	–
Z. multiflora-F	*S. aureus*	0.25 ± 0.19	0.25 ± 0.67	14.65 ± 1.3	15.14 ± 1.28
	E. coli	0.24 ± 0.14	0.25 ± 0.47	11.23 ± 1.3	15.13 ± 1.28
	C. albicans	0.12 ± 0.18	0.17 ± 0.60	9.28 ± 1.28	21.64 ± 1.28
	A. niger	0.11 ± 0.18	0.22 ± 0.60	6.20 ± 1.28	10.40 ± 1.28
	P. aeruginosa	3.50 ± 0.19	9.00 ± 0.67	–	–
Z. multiflora-J	*S. aureus*	0.22 ± 0.19	0.21 ± 0.67	20.90 ± 1.28	21.80 ± 1.28
	E. coli	0.22 ± 0.19	0.25 ± 0.67	16.70 ± 1.30	18.14 ± 1.28
	C. albicans	0.11 ± 0.18	0.20 ± 0.60	16.38 ± 1.30	38.60 ± 1.28
	A. niger	0.11 ± 0.18	0.20 ± 0.6	20.31 ± 1.30	31.80 ± 1.28
	P. aeruginosa	3.50 ± 0.19	4.50 ± 0.67	–	–

MIC = minimal inhibitory concentration; MLC = minimal lethal concentration.
[a] No effect.

Table 3
Antimicrobial activity of thyme oils regardless of type of microorganism.

Plant	Inhibition zone diameter (mm)	MIC (μL/mL)	MLC (μL/mL)
T. vulgaris-K	$9.55^{g[a]}$	0.70^{cd}	$1.^{a}0^{abcd}$
T. vulgaris-SH	19.70^{b}	0.27^{a}	0.41^{a}
T. vulgaris-Y	12.70^{de}	0.79^{cd}	1.20^{abcd}
T. daenensis-K	19.10^{b}	0.65^{cd}	0.99^{abcd}
T. daenensis-SH	13.60^{d}	1.30^{e}	1.80^{f}
T. ksotchyanus-SE	11.20^{f}	0.81^{d}	1.20^{abcd}
T. ksotchyanus-SH	17.50^{c}	0.37^{ab}	0.64^{a}
T. ksotchyanus-Y	7.10^{h}	1.20^{e}	1.70^{ef}
T. fedtschekoi-SE	17.20^{c}	0.65^{cd}	0.77^{abc}
T. pubescens-SE	12.10^{ef}	0.26^{a}	0.75^{ab}
Z. multiflora-SH	12.40^{dfe}	0.54^{bc}	0.83^{abc}
Z. multiflora-J	12.96^{de}	0.69^{cd}	1.57^{def}
Z. multiflora-F	23.10^{a}	0.76^{cd}	0.99^{abcd}

MIC = minimal inhibitory concentration; MLC = minimal lethal concentration.
[a] Lower case superscripts indicate significant differences at $p \leq 0.05$, where [a] is the most sensitive compound followed by [b,c,d,e,f,g].

para-cymene (Kiskó and Roller, 2005; Ultee et al., 2002) and the antagonistic effects of thymol and carvacrol (Gallucci et al., 2009). In other words, a high combination of thymol and carvacrol is not necessarily a criterion for higher antimicrobial effects.

Essential oils are good sources of antimicrobial agents and thyme oils have been promoted as valuable antimicrobial agents among the essential oils (Bagamboula et al., 2004; Burt, 2004). The antimicrobial effects of thymol or carvacrol have been reported against microorganisms (Cosentino et al., 1999; Kim et al., 1995). Essential oil with a high amount of carvacrol has higher antimicrobial activity, with carvacrol identified as a fatal compound for microorganisms as it interferes with membrane functions, increases the lipid membrane permeability and cellular adenosine triphosphate and finally causes death in the cell (Ultee et al., 1999; Vardar-Ünlü et al., 2003).

The antimicrobial effects of carvacrol methyl ether and carvacrol acetate were lower than those of carvacrol (Ben Arfa et al., 2006). Thus, determining the role of each component of thyme essential oils and their antimicrobial effects is impossible and identification

of the role of each component in determining antimicrobial activity needs suitable software that can draw the matrices of these interactions.

Conflict of interest

There is no conflict of interest.

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