**Original Article****A study on the Chemical Composition and Antifungal Activity of Essential Oil from *Thymus caramanicus*, *Thymus daenensis* and *Ziziphora clinopodioides*.**Sepideh Khorasany<sup>1</sup>, Mohamah Hosain Azizi<sup>\*2</sup>, Mohsen Barzegar<sup>2</sup>, Zohreh Hamidi Esfahani<sup>2</sup>

1- PhD Student of Food Science &amp; Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

2- Dept. of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

**Received:** January 2016**Accepted:** March 2016**A B S T R A C T**

**Background and Objectives:** Essential oils (EOs) possess a wide range of significant properties including antiphlogistic, spasmolytic and antinociceptive effects. In this study, we use EOs from *Thymus daenensis*, *Thymus caramanicus* and *Ziziphora clinopodioides*.

**Materials and Methods:** In this study, the plants were collected from the Botany Department, were air dried, grinded and set for extraction with a Clevenger-type apparatus, according to the procedure described in the European pharmacopoeia. *In vitro* antifungal activity of the EOs was evaluated according to Agar dilution method by determining the radial growth rate and inhibition ratio (%).

**Results:** Among the three EOs, *T. daenensis* contains the highest level of thymol (77.62%). *Ziziphora clinopodioides* contains pulegone (31.21%), menth-3-en-8-ol (23.82%), menthol (7.21%), borneol (2.25%), carvacrol (5.38%) and piperitone (5.55%). Only the 9  $\mu$ L concentration of *Z. clinopodioides* EO can prevent mycelium growth of both fungi for 7 days. *Thymus caramanicus* contains carvacrol (65.52%), p-cymene (13.21%), gamma-terpinene (4.44%), thymol (4.14%) and linalool (2.63).

**Conclusions:** According to the results, that compound thymol is more effective than carvacrol in preventing the growth of fungi.

**Keywords:** Essential oils, *Aspergillus flavus*, *Aspergillus parasiticus*, Natural antifungal

**Introduction**

Essential oils (EOs) possess a wide range of significant properties including antibacterial, anti-fungal and antioxidant activities. Furthermore, they exert immunomodulant, psychotropic, acaricide and expectorant effects (1). Due to their multifunctionality, EOs have a vast application in medicine and aromatherapy.

Numerous studies have been undertaken to identify the effect of different food additives, preservatives, chemicals and environmental conditions on the efficient inhibition of fungal growth. Meanwhile, considerable pressure from consumers to decrease or eliminate chemically synthesized additives in their foods has resulted in a

renewal of scientific interest in natural substances (2, 3).

Among thousands of natural constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose or might reasonably be expected to pose a serious threat to human health at current low levels of consumption when utilized as flavoring substances (4). Most of these inhibitors extracted from plants are phenyl propanoids, terpenoids and alkaloids (5).

A novel method to decrease the proliferation of microorganisms and/or their production of toxins is using EOs, which are blends of various lipophilic and volatile substances such as monoterpenes,

\*Address for correspondence: Mohamah Hosain Azizi, Prof, Dept. of Food Science and Technology, Faculty of Agriculture Tarbiat Modares University, Tehran, Iran. E-mail address: Azizit.m@modares.ac.ir

sesquiterpenes, and/or phenyl propanoids that emit a pleasant odor. Moreover, they are considered a part of the performed defense system of higher plants (6).

Kerman Province located at 1,766 meters above the mean sea level is one of the most significant areas for production of medicinal plants. More than 300 types of medicinal plants of great significance are produced in this region. Such medicinal plants include *Thymus daenensis*, *Thymus carmanicus*, and *Ziziphora clinopodioides*, which are used for flavoring and for the treatment of tympanites, coughing, spasm, fever and insomnia. They are also used as appetizers, mouthwash and in the alleviation of toothache. The specific locations where these medicinal plants grow make them quite unique (7, 8).

*Thymus* oils and extracts are broadly utilized in pharmaceutical, cosmetic and perfume industries as well as for flavoring and preservation of various food products (9). This research paper aims to study the EO composition of these species at the latest developmental stage and its coherence with antifungal activity. Because these EOs are native to Kerman province and their composition is very different so that all of them are effective in elimination of fungi. These essential of plants are new products that are very useful for modern agriculture.

## Materials and Methods

### Experimental section

**Plants and materials:** The plants used in this study were collected from the Botany Department, Kerman Center for Research of Agricultural Science and Natural Resources. The samples were air dried, grinded and set for extraction. The reagents used for the study were obtained from the Merk® (Germany).

**Essential oil isolation:** EOs were isolated by water distillation for 3 hours from air-dried material using a Clevenger-type apparatus, according to the procedure described in the European pharmacopoeia (10). Then they were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis. The oils were yellow in color and emitted a distinct sharp odour.

**Gas chromatography - mass spectrometry (GC-MS):** Analyses were undertaken using an Agilent 6890 gas chromatograph fitted with a capillary column (BPX5-5% phenyl polysilphenylene siloxane 30 m × 0.25 mm, film thickness of 0.25 μm),

interfaced with a selective detector 5973 (Agilent Technologies, Palo Alto, CA, USA) operated by HP Enhanced ChemStation Software, version A.03.00. GC parameters were interface temperature of 240°C and MS source temperature stood at 300°C while the MS quadrupole temperature was 220°C. Ionization energy was 70 eV and the scan range was 40-550 u. Helium was employed as the carrier gas at a flow rate of 0.5 ml/min. The GC column oven temperature was raised from 50 to 300°C at a rate of 3°C/min with a final hold time of 5 min. The temperature of injector chamber was maintained at 290°C (11).

**Anti-fungal activity:** *In vitro* antifungal activity of the EOs was evaluated according to Agar dilution method by determining the radial growth rate and inhibition ratio (%). *Aspergillus flavus* PTCC 5004 and *Aspergillus parasiticus* PTCC 5286 were used for the experiment. So as to assess the radial growth rate of strains, the maximum diameter of colonies was measured after 24 hours for 14 days. The inhibition ratio was estimated by Equation 1:

$$\text{Inhibition ratio (\%)} = \frac{C-E}{C} \times 100 \quad (\text{Eq. 1})$$

Where, C is the diameter of mold colony from the control plate, and E is the diameter of the mold colony growth in the experiment plate containing the EO.

Antifungal activity on fungal colony development was obtained by the Agar dilution method (3, 5, 7 and 9 μL) of EO for 100 mL media-PDA. The oils were dissolved in 5% of tween 20 and added to 20 mL of PDA before solidification in Petri dishes (12).

**Statistical analysis:** The experiments were conducted in a completely randomized design (CRD) with different factors and four replications ( $n=4$ ). To assess the effect of herbal extracts and determine the most appropriate concentration during the test days, a factorial split plot was conducted in four replications. Time as the major factor was considered at 14 levels (day 1 to 14) while the minor factor, (herbal extracts), was considered at 3 levels (*T. daenensi*, *Z. clinopodioides* and *T. carmanicus*). Concentration of the extracts was also considered at 4 levels (3, 5, 7 and 9 μL) for determining the most efficient extract concentration. The results obtained were subjected to analysis of variance (ANOVA) using the SAS Statistical Program. Significant differences among the

mean values of treatments were compared using Duncan's test method at  $p \leq 0.01$ .

## Results

Medicinal plants are usually studied for their antifungal compounds. Some researchers have shown that EOs from garlic and onions are able to inhibit the development of *Aspergillus*, *fusarium* and *Penicillium* species (12, 13).

*T. daenensi*, *Z. clinopodioides* and *T. carmanicus* are among the medicinal plants that grow in Kerman Province. Their medicinal application has encouraged more researches in this field. This study attempts to determine the growth inhibition percentage of the EOs of these three plants against *A. flavus* and *A. parasiticus* for 14 days. The percentage of constituents in each EO was analyzed. The medicinal properties of the above plants are reported in Table 1.

**Table 1 .** Medicinal applications of plants

Name of the plant	Effective material	Application
<i>Thymus daenensis</i>	Thymol, carvacrol	Nutraceutical herbal medicine health supplement
<i>Ziziphora clinopodioides</i>	Pulegone, menth-3-en-8-01	vighur's medicine, hyper tension
<i>Thymus carmanicus</i>	Carvacrol p-cymene	Antibacterial, antimycotic, anti-oxidative

A split factorial plot in four trials was designed to analyze the effect of essential oils on preventing the *Aspergillus* fungi growth, and determine the most proper extract concentration during the study. Time, as the major factor, in 14 levels (the 1<sup>st</sup> day to the 14<sup>th</sup>), essential oil, as the minor factor, in 3 levels (*Thymus carmanicus*, *Ziziphora clinopodioides* and *Thymus daenensis*), and the herbal EOs concentration at 4 levels (3, 5, 7, and 9  $\mu$ L) were classified so as to determine the most proper extract concentration.

The results obtained from the functional analysis of variance showed a significant one-percent increase brought about by duration, EOs, and concentration. Moreover, all reciprocal effects including the reciprocal effect of duration on the extract, duration on concentration, essential oils on concentration, and

reciprocal effect of duration on the EO concentration proved to have a significant increase by 1% (Tables 2 and 3).

**Table 2.** Analysis variance of the inhibitory growth rate of *Aspergillus flavus*

Source	df	Mean Square
Day	13	10998.516**
Error	42	.307
Essential oil	2	132304.507**
Concentration	3	71689.108**
Day $\times$ Essential oil	26	705.184**
Essential oil $\times$ Concentration	6	12992.355**
Day $\times$ Concentration	39	317.177**
Day $\times$ Essential oil $\times$ Concentration	78	475.406**
Error	462	.132
Total	671	

\*\*Significant at the one percent level. CV =6.76%

**Table 3.** Analysis variance of the inhibitory growth rate of *Aspergillus parasiticus*

Source	df	Mean Square
Day	13	5069.201**
Error	42	.316
Essential oil	2	164399.713**
Concentration	3	116222.485**
Day $\times$ Essential oil	26	546.261**
Essential oil $\times$ Concentration	6	33265.433**
Day $\times$ Concentration	39	306.111**
Day $\times$ Essential oil $\times$ Concentration	78	469.283**
Error	462	.128
Total	671	

\*\*Significant at the 1% level. CV =6.76%

## Discussion

As shown in tables 4 and 5, increasing the concentration of EOs in all three extracts increases the inhibition ratio of both fungi's growth. The effect of EO decreases during the maintenance period such that only 7 and 9  $\mu$ L concentrations of *T. daenensis* in 100 m/L of PDA growth environment can prevent the mycelium growth of both fungi for 14 days (Figures 1 and 2). Tables 4 and 5 show that only 9  $\mu$ L of *Z. clinopodioides* essential oil can prevent the mycelium growth of both fungi for 7 days.

**Table 4.** The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus flavus* in laboratory conditions

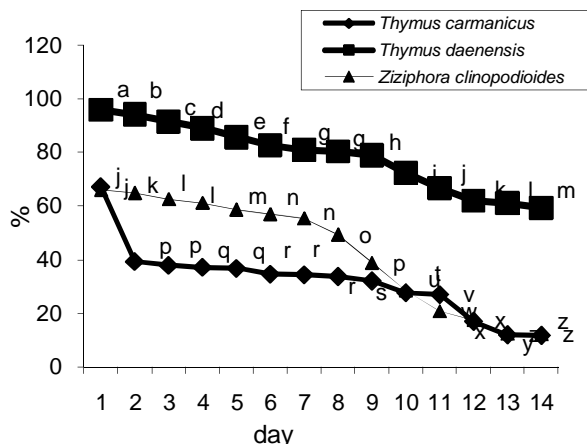
Essential oil	Day	Concentration			
		3 $\mu$ l	5 $\mu$ l	7 $\mu$ l	9 $\mu$ l
<i>Thymus carmanicus</i>	1	35r	37p	100a	100a
	2	31t	37p	41m	51h
	3	31t	35r	39o	49i
	4	28u	34s	38o	49i
	5	28u	34s	37p	48i
	6	27w	29u	37p	46j
	7	27w	29u	36q	46j
	8	26x	28u	36q	45k
	9	25x	27w	35r	43l
	10	0z	27w	31t	41m
	11	0z	26x	31t	40n
	12	0z	25x	20y	31t
	13	0z	0z	0z	25x
	14	0z	0z	0z	20y
<i>Thymus daenensis</i>	1	84b	100a	100a	100a
	2	77c	100a	100a	100a
	3	66d	100a	100a	100a
	4	54g	100a	100a	100a
	5	43l	100a	100a	100a
	6	41m	100a	100a	100a
	7	39o	100	100a	100a
	8	37p	84b	100a	100a
	9	37p	77c	100a	100a
	10	26x	64e	100a	100a
	11	0z	55f	100a	100a
	12	0z	48i	100a	100a
	13	0z	44k	100a	100a
	14	0z	37p	100a	100a
<i>Ziziphora clinopodioides</i>	1	51h	57f	57f	100a
	2	49i	54g	57f	100a
	3	44k	51h	54g	100a
	4	44k	49i	51h	100a
	5	41m	46j	48i	100a
	6	39o	44k	46j	100a
	7	39o	41m	44k	100a
	8	31t	39o	40n	100a
	9	0z	26x	39o	77c
	10	0z	0z	26	77c
	11	0z	0z	15	66d
	12	0z	0z	0z	57f
	13	0z	0z	0z	51h
	14	0z	0z	0z	49i

Similar letters in each column in the level of  $P \leq 0.05$  were not significantly different. Response are shows as mean  $\pm$  standard deviation for 3 replications.

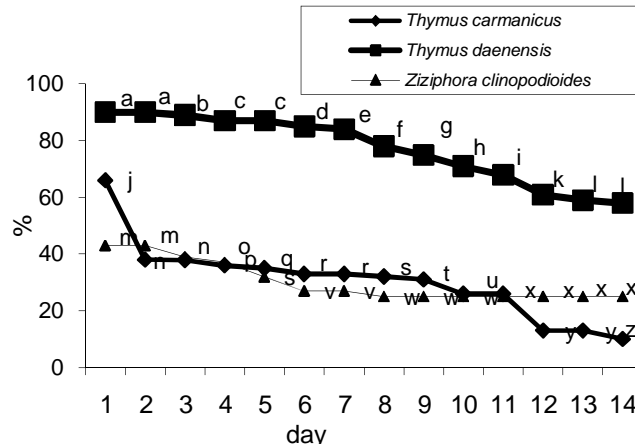
**Table 5.** The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus parasiticus* in laboratory conditions

Essential oil	Day	Concentration			
		3 $\mu$ l	5 $\mu$ l	7 $\mu$ l	9 $\mu$ l
<i>Thymus carmanicus</i>	1	34 q	37 n	100 a	100 a
	2	30 s	34 q	39 m	52 g
	3	28 t	34 q	39 m	51 h
	4	27 u	32 r	37 n	47 i
	5	27 u	32 r	36 o	47 i
	6	26 v	28 t	36 o	44 j
	7	26 v	28 t	36 o	44 j
	8	24 w	27 u	35 p	44 j
	9	24 w	26 v	34 q	42 k
	10	14 y	24 w	30 s	39 m
	11	0 z	24 w	30 s	39 m
	12	0 z	14 y	17 x	30 s
	13	0 z	14 y	16.9 x	24 w
	14	0 z	13.5 y	14 y	17 x
<i>Thymus daenensis</i>	1	63 d	100 a	100 a	100 a
	2	61 e	100 a	100 a	100 a
	3	60.8 e	100 a	100 a	100 a
	4	51 h	100 a	100 a	100 a
	5	51 h	100 a	100 a	100 a
	6	41 l	100 a	100 a	100 a
	7	39 m	100 a	100 a	100 a
	8	37 n	79 b	100 a	100 a
	9	26 v	78.4 b	100 a	100 a
	10	24 w	65 c	100 a	100 a
	11	16 x	58 f	100 a	100 a
	12	0 z	39 m	100 a	100 a
	13	0 z	39 m	100 a	100 a
	14	0 z	35 p	100 a	100 a
<i>Ziziphora clinopodioides</i>	1	17 x	28 t	30 s	100 a
	2	16 x	26 v	29.4 s	100 a
	3	16 x	16 x	26 v	100 a
	4	0 z	0 z	26 v	100 a
	5	0 z	0 z	17 x	100 a
	6	0 z	0 z	0 z	100 a
	7	0 z	0 z	0 z	100 a
	8	0 z	0 z	0 z	100 a
	9	0 z	0 z	0 z	100 a
	10	0 z	0 z	0 z	100 a
	11	0 z	0 z	0 z	100 a
	12	0 z	0 z	0 z	100 a
	13	0 z	0 z	0 z	100 a
	14	0 z	0 z	0 z	100 a

Similar letters in each column in the level of  $P \leq 0.05$  were not significantly different. Response are shows as mean  $\pm$  standard deviation for 3 replications.



**Fig. 1.** The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus flavus* in laboratory conditions



**Fig. 2.** The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus parasiticus* in laboratory conditions

**Chemical composition of EO:** The EOs were analyzed by GC-MS. In total, 35, 33 and 34 constituents were identified and quantified in *T.*

*caramanicus*, *T. daenensis* and *Z. clinopodioides* for 98.31, 97.25 and 98/05% of the total oil composition, respectively (Tables 6, 7 and 8).

**Table 6.** Constituents of *Thymus daenensis*

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
<i>Thymus daenensis</i> Essential oil						
1	8.34	0.02	Hexenal(2E)	860	855	Others
2	11.75	0.07	$\alpha$ - Pinene	934	939	MH
3	12.64	0.05	Camphene	952	954	MH
4	14.66	0.15	Myrcene	992	991	MH
5	15.61	0.03	alpha-phellandrene	1010	1003	MH
6	16.15	0.27	alpha-Terpinolene	1021	1017	MH
7	16.64	1.66	(para) Cymene	1030	1025	MH
8	16.8	0.05	Limonene	1033	1029	MH
9	16.99	1.25	1/8-Cineole	1037	1031	MH
10	18.34	1.18	gamma-Terpinene	1063	1060	MO
11	19.07	0.03	(cis) Sabinene hydrate	1077	1070	MO
12	19.73	0.04	Teroinolene	1090	1089	MH
13	20.54	0.36	Linalool	1106	1097	MO
14	20.69	0.05	(trans) Sabinene hydrate	1109	1109	MO
15	24.40	1.08	Borneol	1183	1169	MO
16	24.75	0.37	Terpinen-4-ol	1190	1177	MO
17	25.54	0.13	(alpha) Terpineol	1206	1189	MO
18	25.68	0.07	Methyl chavicol	1209	1196	MO
19	25.76	0.05	(trans) Dihydrocarvone	1211	1201	MO
20	27.43	0.33	Thymyl methyl ether	1246	1235	Others
21	27.78	0.16	Pulegone	1254	1237	MO
22	30.27	77.62	Thymol	1307	1290	MO
23	30.54	5.99	Carvacrol	1313	1299	MO
24	32.85	0.23	Eugenol	1365	1359	MO
25	33.69	0.13	Geranyl acetate	1384	1381	Others
26	35.52	2.62	Trans- caryophyllene	1427	1419	SH
27	36.32	0.11	Aromadendrene	1446	1441	SH
28	37.07	0.09	alpha-Humulene	1464	1455	SH
29	38.51	0.09	Ledene	1498	1498	SH
30	39.12	0.13	beta-Bisabolene	1514	1506	SH
31	40.42	0.22	cis- alpha- Bisabolene	1546	1507	SH
32	42.15	0.54	Spathulenol	1590	1578	SO
33	42.36	2.08	Caryophyllene oxide	1595	1583	SO

MH: Monoterpene Hydrocarbons; MO: Oxygenated Monoterpenes; SH: Sesquiterpene Hydrocarbons; SO: Oxygenated Sesquiterpenes

**Table 7.** Constituents of *Thymus caramanicus*

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
<i>Thymus caramanicus</i> Essential oil						
1	8.32	0.07	Hexenal(2E)	859	855	Others
2	11.35	0.41	$\alpha$ - Thujene	927	930	MH
3	11.74	0.34	$\alpha$ - Pinene	934	939	MH
4	12.63	0.06	Camphene	952	954	MH
5	14.09	0.08	beta-Pinene	981	979	MH
6	14.35	0.09	octen-3-ol(1)	986	979	Others
7	14.66	0.73	Myrcene	992	991	MH
8	15.61	0.1	Alpha-Phellandrene	1010	1010	MH
9	16.15	0.66	alpha-Terpinolene	1021	1017	MH
10	16.66	13.21	(para) Cymene	1031	1026	MH
11	16.81	0.18	Limonene	1033	1029	MH
12	16.93	0.17	beta-Phellandrene	1036	1030	MH
13	18.35	4.44	gamma-Terpinene	1063	1060	MO
14	19.07	0.38	(cis) Sabinene hydrate	1077	1070	MO
15	19.73	0.06	Teroinolene	1090	1089	MH
16	20.55	2.63	Linalool	1105	1097	MO
17	20.69	0.19	(trans) Sabinene hydrate	1109	1098	MO
18	21.86	0.11	alpha-Necrodol	1132	1132	MO
19	24.40	0.17	Borneol	1183	1169	MO
20	24.74	0.98	Terpinen-4-ol	1190	1177	MO
21	25.54	0.19	(alpha) Terpineol	1206	1189	MO
22	27.00	0.13	Methyl methyl ether	1237	1235	Others
23	27.43	0.13	Carvacrol methyl ether	1246	1245	Others
24	28.09	0.57	Geraniol	1260	1253	MO
25	29.09	0.11	Geranial	1281	1267	MO
26	29.66	4.14	Thymol	1294	1290	MO
27	30.66	65.52	Carvacrol	1316	1299	MO
28	32.39	0.56	(alpha) Terpinyl acetate	1355	1349	Others
29	32.86	0.21	Eugenol	1365	1359	MO
30	35.51	0.92	trans- Caryophyllene	1427	1419	SH
31	37.07	0.05	alpha-Humulene	1446	1455	SH
32	38.51	0.10	beta-Bisabolene	1514	1506	SH
33	42.35	0.62	Caryophyllene oxide	1595	1583	SO

**Table 8.** Constituents of *Zizipora clinopodioides*

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
<i>Zizipora clinopodioides</i> Essential oil						
1	11.75	0.8	$\alpha$ - Pinene	934	939	MH
2	12.64	0.46	Camphene	952	954	MH
3	13.82	0.65	Sabinene	975	975	MH
4	14.09	1.46	beta-Pinene	981	979	MH
5	14.66	0.26	Myrcene	992	991	MH
6	15.21	0.14	3-Octanol	1003	991	Others
7	16.15	0.12	alpha-Terpinolene	1021	1017	MH
8	16.64	0.2	(para) Cymene	1030	1025	MH
9	16.81	0.45	Limonene	1033	1029	MH
10	17	8.39	1/8-Cineole	1037	1031	MO
11	18.34	0.71	gamma-Terpinene	1063	1060	MH
12	19.6	0.99	Menthe- 2.8-diene(para)	1077	1073	MH
13	19.77	0.21	Teroinolene	1090	1089	MH
14	20.54	0.05	Linalool	1106	1097	MO
15	23.29	23.82	Menth-3-en-8-0l(para)	1161	1160	MO
16	24.23	1.78	Menthone(iso)	1166	1163	MO
17	24.43	7.21	Menthol(neo)	1179	1166	MO
18	24.62	2.25	Borneol	1183	1169	MO
19	24.75	1.37	Menthol	1187	1172	MO
20	24.75	0.42	Terpinen-4-ol	1190	1177	MO
21	25.30	0.45	Menthol(iso)	1201	1183	MO
22	25.53	0.33	(alpha) Terpineol	1206	1189	MO
23	26.63	0.66	Shisofurane	1229	1198	MO
24	27.72	31.21	Pulegone	1252	1237	MO
25	28.43	0.44	Piperitone	12.67	1253	MO
26	29.56	0.36	Isobornyl acetate	1291	1286	Others
27	30.11	1.12	Thymol	1303	1290	MO
28	30.51	5.38	Carvacrol	1312	1299	MO
29	32.38	5.55	Piperitenone	1355	1343	MO
30	33.92	0.11	beta-Bourbonene	1389	1388	SH
31	35.51	0.16	trans- Caryophyllene	1427	1419	SH
32	38.14	0.25	Germacrene D	1489	1485	SH
33	38.74	0.04	Bicyclogermacrene	1504	1500	SH
34	42.15	0.24	Spathulenol	1590	1578	SO

MH: Monoterpene Hydrocarbons; MO: Oxygenated Monoterpenes; SH: Sesquiterpene Hydrocarbons; SO: Oxygenated Sesquiterpenes



According to Table 7, *T. caramanicus* contains carvacrol (65.52%), p-cymene (13.21%), gamma-terpinene (4.44%), thymol (4.14%) and linalool (2.63). The chemical composition of the EO of this medicinal herb has been studied by Nejad Ebrahimi *et al.* (14). They concluded that it is of similar composition containing bornoel (2%), which was recognized as an antibacterial material against *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The EO of this herb containing carvacrol, thymol, p-cymene and bornoel is a rich source of antioxidants, which can be used as a natural additive in food and pharmaceutical industries (15).

Carvacrol, also recognized as the primary component of *T. caramanicus* EO is considered a biocidal as it causes bacterial membrane perturbations that leads to leakage of intracellular ATP and potassium ions, and ultimately, cell death (16, 17, 18).

The EO of *T. daenensis* contains thymol (77.62%), carvacrol (5.99%), trans-caryophyllene (2.62%) and caryophyllene oxide (2.08%), and thus possesses antibacterial and antifungal activities. Alavi *et al.* (2010) studied the effect of heat on the chemical composition of EO and indicated that this oil contains thymol (54.7%), carvacrol (5%), linalool (1.9%), p-cymene (11.3%), g-terpinene (12.9%) and b-caryophellene (2.6%), and can be used as herbal medicine, health supplement and nutra-ceutical substance (19). The difference in constituents depends on the climate of the region they are grown, and it is very important.

*Zizipora clinopodioides* contains pulegone (31.21%), menth-3-en-8-0l (23.82%), menthol (7.21%), borneol (2.25%), carvacrol (5.38%) and piperitone (5.55%). Senejoux *et al.* (2012) studied the extract of this herb, and concluded that it can be used for medicinal purposes (20). EOs rich in compounds such as carvacrol are broadly reported to possess high levels of antimicrobial activity (11).

Several studies have focused on the antimicrobial activity of the EOs of thyme so as to identify the responsible compounds (21, 22, 23). Variation in the chemical composition of EOs, in particular, and extracts of medicinal plants may be observed due to the origin, the environmental conditions, and the

developmental stage of collected plant materials. Antimicrobial activity of an EO is attributed mainly to its major components, though the synergistic or antagonistic effect of a compound in minor percentage of the mixture has to be considered (21). Therefore, antimicrobial, antioxidant, and other biological activities may vary, based on the variations in the chemical composition.

However, it was also considered that minor components, as well as a possible interaction between the substances could also affect the antimicrobial activities. In fact, other constituents, such as gamma-terpinene, have been considered to display relatively good activity due to their possible synergistic or antagonistic effects, which is in agreement with our results showing that low amounts of gamma-terpinene during the flowering phase may justify the low antimicrobial activity during this period (11).

### Conclusion

As per the results reached in this work, all studied EOs possess antifungal property against *A. flavus* and *A. parasiticus*. The highest rate of inhibition was observed in *T. daenensis* in concentrations above 7  $\mu$ l in 100 ml PDA media in which no growth was observed for 14 days.

Among the three EOs, *T. daenensis* contains the highest level of thymol (77.62%). Although *T. caramanicus* contains 65.52 % carvacrol, its inhibition rate does not reach 100% at any of the concentrations, indicating that thymol is capable of inhibiting fungal growth completely (100%) for 14 days, and is more effective than carvacrol.

**Authors' contributions:** MHA participated in all processes of the study. SKH contributed in the design of the study and performed the statistical analysis. MB carried out the method of GC-MS and helped to do it. ZHE participated in its design and coordination, and helped to draft the manuscript.

### Financial disclosure

The authors declare that they have no competing interests.

### Funding/Support

This research was done with the financial support by Tarbiat Modares University, Tehran, Iran.

## References

- Pisseri F, Bertoli A, Pistelli L. Essential oils in medicine: Principles of therapy. *Parasitologia* 2008; 50: 89-91.
- Nychas G J E. Natural antimicrobial from plants. In: *New Methods of Food Preservations*. Gold GW. Glasgow, UK 1995; 58-89.
- Bluma R V, Etcheverry M G. Application of essential oils in maize grain: Impact on *Aspergillus section Flavi* growth parameters and aflatoxin accumulation. *Food Microbiol.* 2008; 25: 324-334.
- Rasooli I, Fakoor M H, Yadegarinia D, Gachkar L, Allameh A A, Rezaei M B. Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. *Int. J. Food Microbiol.* 2007; 29: 135-139.
- Holmes R A, Boston R S, Payne G A. Diverse inhibitors of aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol.* 2008; 78: 559-572.
- Reichling J, Saller R, Schnitzler P, Suschke U. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties. *Forsch Komplementmed* 2009; 16:79-90.
- Jalas J. *Thymus* in K.H. Rechinger (Ed). *Flora Iranian* (No.150 ). Springer. New York 1982; 536-538.
- Stahl-Biskup E, Saez F. *Thyme, The genus Thymus*. Taylor and Francis 2002; 331.
- Bauer KD, Garbe H, Surburg H. *Common fragrance and flavor materials*. Wiley-VCH Verlag. Weinheim, Germany 1997.
- Council of Europe. *European Pharmacopeia*, 3<sup>rd</sup>, ed. Strasbourg 1997.
- Baydar H, Karadogan G, Ozcan G, Sagdic O. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja Species* with commercial importance in Turkey. *Food Cont.* 2004; 15:169-172.
- Benkeblia N. Antimicrobial activity of essential oil extract of various onions (*Allium Cepa*) and garlic (*Allium Sativum*). *Lebensmittel-Wissenschaft and Technologie* 2004; 37: 263-268.
- Yin M C, Tsao S-M. Inhibitory effect of seven *Allium* plants upon their *Aspergillus* species. *J Food Microbiol.* 1999; 49: 49-56.
- Nejad Ebrahimi S, Hadian J, Mirjalili M H, Sonboli A, Yousefzadi M. Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phonological stages. *Food Chem.*2008; 110:927-931.
- Safaei-Ghomi j, Ebrahimabadi AH, Djafari-Bidgoli Z, Batooli H. GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol. *Food Chem.* 2009; 115 (4): 1524-1528.
- Helender I M, Alakomi H L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid E J. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J Agri. Food Chem* 1998; 46: 3590-3595.
- Juven BJ, Kanner J, Schued F, Weisslowicz H. Factors that interact with the antibacterial action of *Thymus* essential oil and its active constituents. *J Appl. Bacteriol.* 1994; 76: 626-631.
- Ultee A, Kets E P W, Smid E J. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* 1999; 65: 4606-4610.
- Alavi S S, Ferdosi M, Jannatifard F, Eslami M, Alaghemandan H, Setare M. Behavioral addiction versus substance addiction: Correspondence of psychiatric and psychological views. *Int. J. Prevent. Med.* 2012; 3 (4):290-294.
- Senejoux F, Girard C, Aisa H A, Bakri M, Kerram P, Berthelot A, Bevalot F, Demougeot C. Vasorelaxant and hypotensive effects of a hydroalcoholic extract from the fruits of *Nitraria sibirica* Pall. (Nitrariaceae). *J. Ethnopharmacol.* 2012; 141(2): 629-634.
- Burt S. Essential oils; their antimicrobial properties and potential applications in foods-a review. *Int. J. Food Microbiol.* 2004; 94: 223-253.
- Crespo ME, Gomis E, Jimenez J, Navarro C. Antibacterial activity of the essential oil of *thymus serpylloides* subspecies *gadorensis*. *Microbios* 1990; 61: 181-184.
- Nelson RR. *In vitro* activities of five plant essential oils against methicillin-resistant *staphylococcus aureus* and vancomycin-resistant *Enterococcus Jaeciumz.* *J. Antimicrobial. Chemothe.* 1997; 40:305-306.