Acta Scientific Agriculture (ISSN: 2581-365X)

Volume 2 Issue 5 May 2018

Research Article

A Study on Chemical Composition and Antifungal Activity of Essential Oil from *Thymus caramanicus, Thymus daenensis* and *Ziziphora clinopodioides*

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Received: March 12, 2018; **Published**: April 12, 2018

Abstract

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

Purpose of the Study: This study attempts to determine the growth inhibition level of the essential oils of three plants against *Aspergillus flavus* and *Aspergillus parasiticus* for 14 days.

Results: The highest rate of inhibition was observed in *Thymus daenensis* in concentration above 7 µL in 100 mL of PDA medium in which no growth was observed during 14 days.

The Main Findings: Among the three essential oils, *T. daenensis* contains the highest level of thymol (77.62%). *Ziziphora 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%). Only a concentration of 9 µL of essential oils of *Z. clinopodioides* 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).

Conclusion: Although *T. caramanicus* contains 65.52% carvacrol, its inhibition growth ability does not reach 100% in all concentrations and it is capable of inhibiting fungal growth completely (100%) at 7 and 9 µL concentrations for one day. This indicates that compound thymol is more effective than carvacrol in prevent of growth fungi.

Keywords: Essential Oils; *Aspergillus flavus; Aspergillus parasiticus*; Natural Antifungal

Introduction

Essential oils (EO_s) 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 multi-functionality, EOs has a vast application in medicine and aromatherapy.

Numerous studies have been undertaken to identify the effects of different food additives, preservatives, chemicals and environmental conditions on 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 thus 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 essential oils, which are blends of various lipophilic and volatile substances such as monoterpenes, 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 m above mean sea level is one of the most significant areas for medicinal plants production. 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 essential oil composition of these species at the latest developmental stage and its coherence with antifungal activity. Because this essential oil are native Kerman province and the composition of these are very different so that every of essential oil is effective in elimination of fungi. These essential of plants are the new products that are very useful for modern agriculture.

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 Merk®.

Essential oil isolation

Essential oils 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]. These oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analyses. 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 30m × 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 Chem Station Software , version A.03.00. GC parameters were; interface temperature of 240°C and MS source temperature stood at 300°C while MS quadrupole temperature was 220°C. Ionization energy was 70 eV and scan range was 40-550 u. Helium wan 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 minutes. The temperature of injector chamber was maintained at 290°C [11].

Anti-fungal activity

In vitro antifungal activity of the essential oils 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:

$$\text{Inhibition ratio (\%)} = \frac{C - E}{C} \times 100 \qquad \text{---Equation 1}$$

where, C is the diameter of mold colony from control plate and E is the diameter of the mold colony growth in the experiment plate containing the essential oil.

Antifungal activity on fungal colony development was obtained by Agar dilution method (3, 5, 7 and 9 µL) of essential oil 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. daenensis, Z. clinopodioides* and *T. caramanicus*). Concentration of 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 $p \leq 0.01$ in the statistical package.

Results

Medicinal plants are usually studied for their antifungal compounds. Some researchers have shown that essential oils from garlic and onions are able to inhibit the development of *Aspergillus, Fusarium* and *Penicillium* species [12,13].

T. daenensis, Z. clinopodioides and *T. caramanicus* are among 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 essential oils of these three plants against *A. flavus* and *A. parasiticus* for 14 days. The percentage of constituents in each essential oil was analyzed. The medicinal properties of the above plants are reported in the following table (Table 1).

Name of the plant	Effective material	Application
Thymus daenasis	Thymol, carvacrol	Nutraceutical herbal medicine health supplement
Ziziphora clinopodioides	Pulegone, menth-3-en-8-01	Vighur's medicine, hyper tension
Thymus caramanicus	Carvacrol p-cymene	Antibacterial, antimycotic, anti-oxidative

Table 1: Medicinal applications of plants.

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 1st day to the 14th), the essential oil, as the minor factor, in 3 levels (*Thymus caramanicus, Ziziphora clinopodioides, Thymus daenensis*), and the herbal essential oils concentration including 4 levels (3, 5, 7, and 9 µL) 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 the duration, essential oils, and the concentrations.

Moreover, all reciprocal effects including the reciprocal effect of the duration on the extract, duration on concentration, essential oils on concentration, and the reciprocal effect of duration on the essential oil concentration proved to have a significant increase by one percent (Tables 2 and 3).

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

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

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

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

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

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

Discussion

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

Essential oil	Day	Concentration			
		3l	5l	7l	9l
Thymus carmanicus	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
Thymus daenensis	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
Ziziphora clinopodioides	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

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

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

Essential oil	Day	Concentration			
		3l	5l	7l	9l
Thymus carmanicus	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
Thymus daenensis	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
Ziziphora clinopodioides	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

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

Similar letters in each column in the level of P ≤ 0.05 were not significantly different. Response shows as mean ± standard deviation for 3 replications.

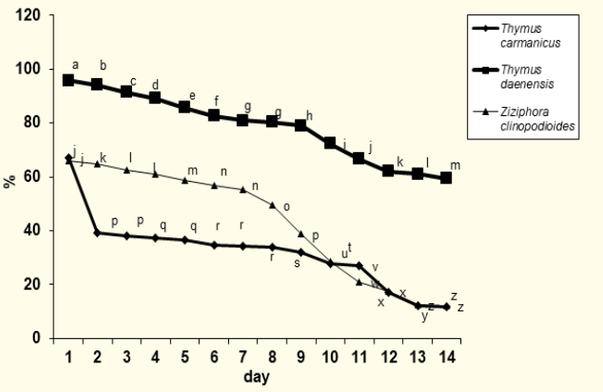

Figure 1: The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus flavus* in laboratory conditions.

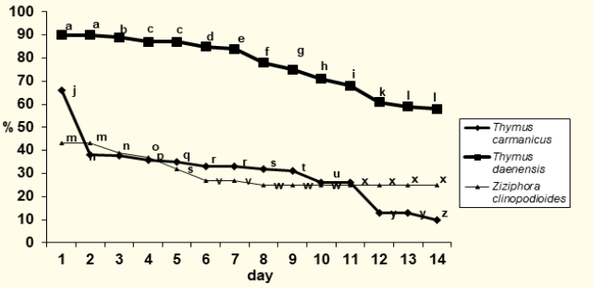

Figure 2: The effect of different concentrations of essential oil on the mean of growth inhibition percentage of *Aspergillus parasiticus* in laboratory conditions.

Chemical composition of the essential oil

The essential oils 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 6-8).

The obtained results indicate that carvacrol constitutes the major portion of *T. caramanicus*, thymol forms the major portion of *T. daenensis* and pulegone and menth-3-en-8-ol (para) constitute the major portions of *Z. clinopodioides*. However, oxygenated monoterpenes, sesquiterpene hydrocarbons, monoterpene hydrocarbons and oxygenated sesquiterpenes were also found as trace or minor components.

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 essential oil 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 essential oil 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].

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
			Thymus daenasis Essential oil			
1	8.34	0.02	Hexenal(2E)	860	855	Others
2	11.75	0.07	- 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

Table 6: Constituents of Thymus daenasis.

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

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
			Thymus caramanicus Essential oil			
1	8.32	0.07	Hexenal(2E)	859	855	Others
2	11.35	0.41	- Thujene	927	930	MH
3	11.74	0.34	- 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 7: Constituents of Thymus caramanicus.

No.	RT	Area% A1	Name	KI Sample	KI Adams	Type
			Ziziphora clinopodioides Essential oil			
1	11.75	0.8	- 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	1681	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

Table 8: Constituents of Ziziphora clinopodioides.

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

Carvacrol, which is recognized as the primary component of *T. caramanicus* essential oils is considered a biocidal as it causes bacterial membrane perturbations that leads to leakage of intracellular ATP and potassium ions and ultimately cell death [16-18].

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

Ziziphora 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].

Essential oils 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 essential oils of thyme so as to identify the compounds responsible [21-23]. Variation in chemical composition of essential oils, 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 essential oil is attributed mainly to its major components, although the synergistic or antagonistic effect of one compound in minor percentage of mixture has to be considered [21]. Therefore, antimicrobial, antioxidant, another 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 study, all studied essential oils possess antifungal property against *A. flavus* and *A. parasiticus.* The highest rate of inhibition was observed in *T. daenensis* in concentrations above 7 µL in 100 mL PDA media in which no growth was observed for 14 days.

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

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