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RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Qualitative, Quantitative and Antimicrobial Activity Variations of the Essential Oils Isolated from *Thymus Vulgaris* and *Micromeria Fruticosa* Samples Subjected to Different Drying Conditions

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Abstract
The treatment of plant materials after collection may affect their volatile components. Three aliquots of *Thymus vulgaris* and *Micromeria fruticosa* representing fresh, freeze- and shade-dried samples were subjected to hydrodistillation to obtain essential oil. Qualitative and quantitative study of the essential oils and their antimicrobial activity was performed. GC–MS analyses are used to identify the oil components based on relative retention indexes and MS comparison through NIST library. Antimicrobial activities against *E. coli*, *P. aeruginosa*, *S. aureus*, *B. cereus* and *C. albicans* were studied. Little decrease in oil contents was observed in freeze-dried plant samples while shade drying resulted in dramatic loss of the oils contents. The percentage of the major components were affected by the various treatment conditions. Applied vacuum in the freeze-dried samples resulted in loss of the more volatile components. Shade drying leads to slow decrease in moisture contents and permits enzymatic activity resulting in large changes in the percentage of some components in *M. fruticosa* such as pulegone and β-caryophyllene. The oil samples from both plants expressed better activity against *P. aeruginosa* and *C. albicans*. The level of antimicrobial activities was equal in all oil samples.

Keywords GC–MS · *Thymus vulgaris* · *Micromeria fruticose* · Essential oil · Drying · Antimicrobial

1 Introduction

Thyme has achieved a reliable place in phytotherapy for the treatment of the upper respiratory tract and bronchial inflammation. The essential oil of thyme is applied as expectorant in cough associated with cold [1]. Essential oil of thyme was reported to have dose-dependant anti-inflammatory effect [2]. Thyme essential oil was reported to have antimicrobial activities [3]. It was also effective against food-related bacteria and fungus [4]. The antimicrobial potential of the essential oil is related to the phenolic compounds mainly, thymol and the terpene hydrocarbon, γ-terpinene [5–7]. The essential oil exhibited in vitro cytotoxic effects against human ovarian adenocarcinoma cells to chemotherapeutic agents as well as a significant antitumor effect in mice [8]. It also possesses strong antioxidant effect [9, 10].

Micromeria fruticosa is a medicinal herb, widely distributed in eastern Mediterranean region. Infusion prepared from the aerial parts is used to treat many diseases including abdominal pains, diarrhoea, eye infections, heart disorders, elevated blood pressure, colds and wounds [11, 12]. The essential oil of *M. fruticosa* exhibited marked activity against fourteen bacteria, three fungi and a yeast and weak antioxidant activity in DPPH and inhibition of linoleic acid oxidation [13]. *M. fruticosa* oil showed marked antitumor activities against human colon tumour cells (HCT) and mammary carcinoma F7 (MCF7) [14]. The essential oil also showed antilipase and antiamylase activity due to higher amount of α-pinene and β-pinene [15].

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The effect of drying on the aromatic components of *T. vulgaris* was attempted. The effect of convective drying, vacuum-microwave drying, freeze-drying and combination of the first two methods were studied [16]. The authors recommended combination of convective drying at 40 °C followed by vacuum-microwave drying at 240 W to obtain the best oil quality based on chemical and sensory analyses [16]. Another study evaluated the effect of freeze-drying and oven-drying at 30 and 60 °C. The effect on the total volatile consents was insignificant under freeze-drying and oven-drying at 30 °C [17]. The effect of sun drying and shade drying on the oil quantity and quality of different organs of *M. barbata* before flowering was also reported [18].

The value of the two plants is related to their essential oil contents. Their use as spices or food preservatives will be greatly affected by the oil percentage. The present work aims to study the effect of different drying methods on both quantity and quality of the essential oils in *T. vulgaris* and *M. fruticose* via hydrodistillation and GC/MS analyses. The impact on the antimicrobial efficacy of the oils will also be evaluated.

2 Materials and Methods

2.1 Plant Material

The aerial parts of *Thymus vulgaris* L. were collected (2.85 kg) from Ma'soum Cir., Zarqa, Jordan, while those of *Micromeria fruticosa* Druce were collected (3.4 kg) from Idna, Al-Khalil city, West Bank. Samples were authenticated by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#7385 and #7642, respectively) was deposited at the herbarium of this centre.

2.2 Preparation of the Oils

The essential oils of all plant samples were obtained by the hydrodistillation method for 5 h using a Clevenger apparatus with 2-L round-bottom flask. The fresh plant material was cut into small pieces about 2 cm long and directly utilized for oil isolation. Intact plant material was spread on the Lyophilizer tray or laboratory floor for freeze or shade drying, respectively. The dried plant materials were cursed and utilized for oil isolation. After separation of the oil layer, the condensate was extracted with ether. The ether extract was added to the oil, dehydrated over anhydrous sodium sulphate and evaporated under reduced pressure (350 m bar) to obtain the essential oil. Experiments were performed in triplicates.

Each plant was divided into three equal parts. The first parts of each plant were directly subjected to hydrodistillation. The second parts were subjected to freeze-drying in a tray type Lyophilizer (MILLROCK, STELLAR® Laboratory Freeze Dryer, Model NO. LD85B3-I) followed by hydrodistillation. The third part of each plant was left for drying in shade at well-ventilated controlled room temperature (22 ^{0}C). After complete drying (10 Days) the plant materials subjected to hydrodistillation. Experiments were performed in triplicate. The average quantities and percentage, relative to the original fresh plants weights, of essential oil from each sample are presented in Table 1.

2.3 GC/MS Analysis

Aliquots of diluted oils in methanol (1 uL of 5 ppm concentration) were then injected into the GC/MS apparatus autosampler. Samples were injected by split-less mode. Analysis was performed using GC/MS (Agilent Model 7890 MSD) equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm coating). Temperature program started with 70 °C for 5 min and programmed at the rate of 5 °C/min to 290 °C, then held isothermally for 5 min. The detector and injector temperatures were adjusted to 290 and 280 °C, respectively. Helium (99.999% purity) was the carrier gas at a flow rate of 1.0 mL/min. In addition to significant quadrupole MS operating parameters: Electrospray ionization at 70 eV with scan mass range of 30 to 600 m/z. Identification of the components was made comparing their mass spectra with library of the National Institute of Standards and Technology (NIST 2017). The analysis and processing of the results were controlled using MASSHUNTER software. The identity of thymol, menthol, pulegone and eugenol was confirmed by co-injections of authentic materials with the oil samples.

2.4 GC Analysis

GC spectra was obtained using the above-mentioned conditions on GC Agilent 7890B, equipped with a HP-5 19091 J-413 capillary column (30 m × 0.25 mm). FID detector was used and identification of peaks achieved by comparing the relative retention index (RRI) to a series of n-alkanes. The quantitative determination of each compound was performed based on computerized peak area measurements obtained from the samples injected via autosampler (Tables 2, 3).

2.5 Antimicrobial Activity

2.5.1 Bacterial Strains

The antimicrobial assays were performed using reference strains of the American Type Culture Collection (ATCC) and National Collection of Type Culture (NCTC) available in

Table 1 Samples and essential oil weights of different *T. vulgaris* and *M. fruticosa* samples*

Plant name	Fresh Sample			Freeze-dried sample			Shade-dried sample		
	Sample (g)	Oil (g)	Oil %**	Sample (g)	Oil (g)	Oil %**	Sample (g)	Oil (g)	Oil %**
T. vulgaris	295	0.426 ± 0.017	1.44	57 ± 0.96	0.380 ± 0.011	1.29	51 ± 0.75	0.105 ± 0.004	0.04
M. fruticosa	362	1.020 ± 0.025	0.28	92 ± 1.01	0.970 ± 0.021	0.27	86 ± 0.98	0.250 ± 0.008	0.07

*Values are mean of triplicate determination (n = 3) ± standard deviations
**Percentage were calculated based on the weights of the fresh plant samples

Table 2 Essential oil components of different *T. vulgaris* samples

No	Name	RT	RI measured	RI Reported	Area %		
					Fresh	Freeze-dried	Shade-dried
1	3-Octanol	9.3888	984	993.2	0.32	0.40	0.38
2	α-Terpinene	11.2841	1009	1017.1	0.14	0.34	0.38
3	*p*-Cymene **(1)**	12.0992	1014	1024.3	6.57	11.03	15.91
4	Eucalyptol	12.4743	1032	1031.8	0.25	0.13	0.42
5	γ-Terpinene **(2)**	13.8845	1050	1059.7	4.35	8.70	7.74
6	*cis*-Sabinene hydrate	14.5054	1052	1541.1	0.04	0.26	0.37
7	α-Terpineol	18.3790	1179	1189.7	0.14	0.19	0.27
8	Estragole	20.7089	1190	1195.8	0.31	–	0.32
9	4,7-Dimethylbenzofuran **(3)**	21.2458	1224	1220	2.20	1.48	2.25
10	Thymol **(4)**	24.6548	1291	1290.1	75.87	68.80	53.96
11	β-Caryophyllene **(5)**	28.7947	1419	1420.1	1.74	2.36	3.94
12	Unidentified	29.8944	1486	1472	0.27	0.18	0.72
13	Bicyclogermacrene	31.2657	1495	1494.1	0.25	0.51	1.05
14	4-Ethoxy ethylbenzoate	32.1325	1522	1521	0.26	0.19	0.57
15	Spathulenol	33.6979	1577	1576.4	0.19	0.17	0.46
16	β-Caryophyllene oxide	33.8597	1574	1580.6	0.16	0.15	0.15
17	τ-Cadinol	35.5221	1649	1637.8	0.27	0.04	0.82
18	Retinoic acid	47.8772	2352	–	0.11	0.13	0.47
	Total				93.17	94.88	89.46

the Microbiology Laboratory of College of Pharmacy/Prince Sattam University (Al-Kharj- Arabia Saudi). The standard microbial strains used were as follows: *E. coli* (ATCC 11,209), *P. aeruginosa* (ATCC 10,145), *S. aureus* (NCTC 6571), *B. cereus* (ATCC 10,875) and *C. albicans* (ATCC 14,053). Tested strains were routinely grown aerobically at 37^0C. Microbial suspension of equivalent to a 0.5 McFarland standard was used.

2.5.2 Antimicrobial Assay

Antimicrobial assays for MIC and MMC were accomplished using the reported broth dilution method following the Clinical and Laboratory Standards Institute guidelines [19]. Starting from solution of 10 mg/ml from each oil sample, serial dilutions were prepared and tested in the ranged from 3.125 µg/ml to 50 µg/ml. Ten µl of tested microbes were inoculated to each concentration. Mueller–Hinton broth (MHB, Scharlau) alone was tested as sterility control, and untreated microbes inoculated on MHB alone and with different concentration of the solvent DMSO were tested as a negative control. After 24 h incubation at 37^0C, the lowest concentration of antimicrobial agent that inhibited visible growth MIC was determined. Minimal microbicidal concentration (MMC) was determined by subculturing 10µL from the broth dilution in Mueller–Hinton agar for bacteria (MHA, Scharlau) and Sabouraud dextrose agar (SDA, Scharlau) for *C. albicans.* MMC is the minimum concentration of an oil that kills 100% of microbes after 24 h incubation. All assays were carried out in triplicates to confirm the results.

Table 3 Essential oil components of different *M. fruticosa* samples

No	Components	RT	RI measured	RI reported	Area %		
					Fresh	Freeze-Dried	Shade-Dried
1	β -Pinene	8.7809	973	977.7	0.10	0.13	0.01
2	3-Octanol	9.3878	984	993.2	0.35	0.31	0.60
3	D-Limonene	11.9956	1018	1029.5	0.55	0.13	0.14
4	Eucalyptol	12.4741	1032	1031.8	0.15	0.21	0.05
5	β-Ocimene	12.7136	1048	1047.7	0.12	0.34	0.13
6	*p*-Mentha-3,8-diene **(6)**	14.5831	1071	1060	2.23	2.22	2.49
7	α-Terpinolene	15.3787	1088	1086.9	0.56	0.64	0.95
8	6-*cis*-Allocimene	17.6621	1131	1129.9	0.10	0.15	0.10
9	3,9-Epoxy-1-*p*-menthene	18.1473	1144	1178	0.39	0.34	0.34
10	α-Terpineol **(7)**	18.5095	1175	1189.7	3.82	3.56	1.56
11	*l*-Menthone **(8)**	18.6971	1164	1159.1	19.54	17.47	20.40
12	Borneol	18.9302	1167	1166.2	–	–	0.89
13	Pinocamphone	19.3634	1170	1172.8	0.84	0.74	0.53
14	Isopulegone	19.6933	1177	1176.6	1.79	2.32	2.50
15	*dl*-Menthol **(9)**	20.0197	1174	1177.3	35.47	36.01	33.19
16	(-)-*cis*-Isopiperitenol	20.8576	1203	1228	0.06	0.02	0.23
17	4,7-Dimethyl benzofuran	21.2393	1244	1220	0.51	0.20	0.39
18	Pulegone **(10)**	22.4360	1237	1234.3	23.70	22.14	13.79
19	γ-Elemene	26.0004	1340	1337	0.10	0.18	0.22
20	Piperitenone	26.0908	1340	1340.7	0.41	0.42	0.06
21	Eugenol	26.7117	1366	1357.8	0.43	0.04	0.01
22	α-Copaene	27.3327	1376	1376.2	0.05	0.09	0.31
23	β-Elemene	27.9084	1391	1390.4	0.04	0.08	0.15
24	Jasmone	28.1219	1394	1394.6	0.19	0.16	0.02
25	β-Caryophyllene **(5)**	28.8011	1419	1420.1	3.82	5.94	13.97
26	Aromandendrene	29.4156	1440	1447	0.02	0.06	0.12
27	Isogermacrene D	29.6032	1448	1451	0.04	0.08	0.14
28	1,4,7,-Cycloundecatriene	29.8878	1481	1472	0.19	0.31	0.59
29	γ-Muurolene	30.6511	1447	1476.2	0.08	0.19	0.19
30	*Cis*- β-Copaene	30.7805	1436	1433.1	0.42	0.87	1.44
31	(+)-Bicyclogermacrene	31.2657	1495	1494.1	0.42	0.73	0.85
32	α-Muurolene	31.3886	1499	1498.3	0.03	0.04	0.12
33	α-Cadinene	32.1066	1538	1533.3	0.22	0.32	0.62
34	β-Caryophyllene oxide	33.8661	1574	1580.6	0.12	0.12	0.04
	Total				96.86	96.56	97.14

2.5.3 Data Analysis

Values of three replicates are presented as mean ± standard deviation. The statistical parameters applied were the Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

3 Results and Discussion

3.1 Preparation of the Oil and GC–MS Study

The percentage of the essential oils were calculated relative to the fresh plants samples used. The essential oil average yield resulted from the three experiments utilizing the two plants was higher for the fresh samples followed by the freeze-dried samples, while the shade-dried samples gave the lowest

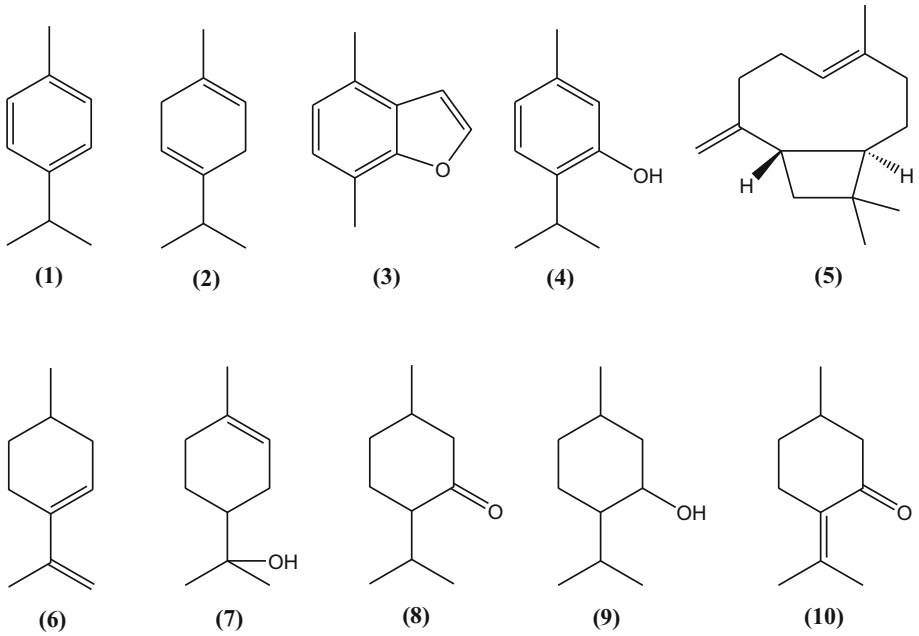

Fig. 1 Major chemical components of the essential oils of *T. vulgaris* and *M. fruticosa* samples

Table 4 MIC and MMC of different *T. vulgaris* and *M. fruticosa* oils VS tested microbes

Samples	*T. vulgaris*					
	Fresh		Freeze-dried		Shade-Dried	
	MIC	MMC	MIC	MMC	MIC	MMC
E. coli ATCC 11,209	12.5	18.25	12.5	18.25	12.5	18.25
P. aeruginosa ATCC 10,145	9.125	12.5	9.125	12.5	9.125	12.5
S. aureus NCTC 6571	18.25	25	18.25	25	18.25	25
B. cereus ATCC 10,875	25	36.5	25	36.5	25	36.5
C. albicans ATCC 14,053	9.125	12.5	9.125	12.5	9.125	12.5
Samples	*M. fruticosa*					
	Fresh		Freeze-Dried		Shade-Dried	
	MIC	MMC	MIC	MMC	MIC	MMC
E. coli ATCC 11,209	12.5	18.25	12.5	18.25	12.5	18.25
P. aeruginosa ATCC 10,145	9.125	12.5	9.125	12.5	9.125	12.5
S. aureus NCTC 6571	18.25	25	18.25	25	18.25	25
B. cereus ATCC 10,875	18.25	25	18.25	25	18.25	25
C. albicans ATCC 14,053	9.125	12.5	9.125	12.5	9.125	12.5

amount of oils. These findings were in agreement with previous studies on *T. vulgaris* and *M. barbata* [16–18]. The obtained results indicated that the oil is lost to some extent by the effect of vacuum applied during freeze-drying. Shade drying process resulted in greater loss of the essential oil. The fresh samples oil yield was 1.44%, decreased to 1.29% in the freeze-dried *T. vulgaris* samples, while the oil percentage in case of *M. fruticosa* decreased from 0.28 in the fresh samples to 0.27% in the freeze-dried samples. Shade drying of samples resulted in dramatic loss of the oils to reach 0.04 and 0.07% in *T. vulgaris* and *M. fruticose,* respectively (Table 1).

The study of the oil composition by GC–MS revealed little differences in the number of compounds (Fig. 1) among the different treated samples. The GC chromatogram of *T. vulgaris* oil showed the presence of 17 identified components in the fresh and shade-dried samples, while the freeze-dried sample lacks the presence of the minor component estragole (Table (2). *T. vulgaris* fresh sample oil showed five components with concentrations above 2%, namely thymol **(4)** (75.87%), *p*-cymene **(1)** (6.57%), γ-terpinene **(2)** (4.35%), 4,7-dimethylbenzofuran **(3)** (2.20%) and *β*-caryophyllene **(5)** (2.32%)(Table 2). Thymol **(4)** was the main component in all samples as previously reported [20]. Freeze-dried samples

expressed the greatest loss in thymol **(4)** where its percentage decreased to 68.80% followed by 4,7-dimethylbenzofuran **(3)** (1.48%), while the percentage of *p*-cymene **(1)**, γ-terpinene **(2)** and β-caryophyllene **(5)** were increased. The shade-dried sample showed an increase in the percentage of β-caryophyllene **(5)** to 3.95% representing more than double its percentage in the fresh sample. Great increase was also observed in the percentage of *p*-cymene **(1)** (15.91%) and γ-terpinene **(2)** (7.74%). Little changes were observed in the amount of 4,7-dimethylbenzofuran **(3)**. On the other hand, thymol **(4)** percentage decreased to 53.96%.

GC–MS analysis revealed the presence of 33 components in both fresh and freeze-dried oil samples of *M. fruticosa*. However, the shade-dried samples showed borneol as extra component (Table 3). Menthol **(9)** represents the major component in the three oil samples of *M. fruticosa* with the highest percentage in the freeze-dried sample (36.01%)(Table 3). The slight increase in menthol **(9)** contents compared with the fresh sample (35.47%) can be explained by the loss in other more volatile components under the effect of the applied vacuum. Loss due to shade drying was about 2% compared with the fresh sample. The second major component pulegone **(10)** undergoes dramatic loss during shade drying (Table 3). In support to our finding the dried samples of *M. frivaldszkyana* expressed considerable reduction in pulegone **(10)** contents [21]. The level of menthone **(8)** was highest in the shade-dried oil sample (20.40%) followed by the fresh oil sample (19.54%) while the freeze-dried oil sample contains 17.47% menthone **(8)**. β-Caryophyllene **(5)** increased by about 10% in the shade-dried sample compared with the fresh sample. These large changes in some components between the fresh and shade-dried oil samples may be due to the enzymatic activity that takes place in the plant leaves before the moisture contents decrease to a level able to stop enzymatic activity. The optimum enzyme activity takes place at 45% moisture contents or more [22]. On the other hand, the freeze-dried sample will not support any enzymatic activity and only changes due to the loss of more volatile components under the effect of the applied vacuum was observed.

3.2 Antimicrobial Activity

As illustrated in Table 4, a difference was observed between Gram-positive comparing to Gram-negative bacteria and *C. albicans* sensitivity to tested samples. *T. vulgaris* and *M. fruticosa* oils showed better activity against *P. aeruginosa* and *C. albicans* where the MIC and MMC were 9.125 and 12.5 µg/ml followed by *E. coli* 12.5 and 18.25 µg/ml, while Gram-positive MIC and MMC were above 18.25 and 25 µg/ml, respectively. The higher MIC and MMC of oils with *B. cereus* may be due to its property as spore forming bacteria. *M. fruticosa* oils showed better activity against *B. cereus* comparing with *T. vulgaris*. The main components of *T. vulgaris* oil, namely thymol (**4**), *p*-cymene (**1**), γ-terpinene (**2**) and β-caryophyllene **(5)**, are reported to have antimicrobial activity [23, 24]. Menthol (9), menthone (8) and pulegone **(10)** detected in high percentage in *M. fruticosa* expressed strong antimicrobial activity [25–27]. The activity of plants oils had no significant difference in both plants irrespective of plant conditions either as normal drying, freeze-drying or fresh. Although there was variation in the chemical composition of the different samples, the antimicrobial activity was equal. This indicates that the observed effectiveness is not dependent on single component but is the result of various components of the oil collectively. It is proposed that the minor components may enhance the effect of the major components [28]. The hydrocarbon p-cymene was proved to have synergistic effect with carvacrol [29].

4 Conclusion

In both cases dramatic loss of the oils was observed in shade drying samples. The freeze-dried sample oil per cent was 1.29 compared to 1.44 in the fresh sample of *T. vulgaris*, while the oil per cent in freeze-dried *M. fruticosa* was about 0.27 slightly less compared with 0.28 in the fresh sample. The components of the various oil samples were more or less the same; however, their percentage changed from fresh to dried sample depending on the different techniques used. Some components in *T. vulgaris* and *M. fruticosa* such as thymol **(4)**, pulegone **(10)** and γ-caryophyllene **(5)** expressed great change in the percentage. The antimicrobial activity of the different oil samples expressed almost equal potencies. The two oil samples showed stronger activity against *P. aeruginosa* and *C. albicans*.

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Declarations

Conflicts of interest The authors declared that there is no conflict of interest.

References

1. Stahl-Biskup, E.; Venskutonis, R.P.: in Handbook of Herbs and Spices (Second Edition), Ed. K.V. Peter, K.V.: Volume 1, Woodhead Publishing Limited (2012)
2. Juhás, Š, et al.: Anti-inflammatory effects of thyme essential oil in mice. Acta. Vet. Brno. **77**, 327–334 (2008)
3. Bhaskara, M.V., et al.: Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry **47**, 1515–1520 (1998)

4. Borugă, O., et al.: *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. J. Med. Life. **7**(3), 56–60 (2014)
5. Dorman, H.J.D.; Deans, S.G.: Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. **88**(2), 308–316 (2000)
6. Skočibušić, M.; Bezić, N.; Dunkić, V.: Phytochemical composition and antimicrobial activities of the essential oils from Satureja subspicata Vis growing in Croatia. Food Chem. **96**(1), 20–28 (2006)
7. Rota, M.C., et al.: Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control **19**(7), 681–687 (2008)
8. AitMbarek, L., et al.: Cytotoxic effect of essential oil of thyme (*Thymus broussonettii*) on the IGROV1 tumor cells resistant to chemotherapy. Braz J Med Biol Res **40**(11), 1537–44 (2007)
9. Grigore, A., et al.: Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. Roman Biotech Lett **15**(4), 5436–5443 (2010)
10. Sacchetti, G., et al.: Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem **91**(4), 621–632 (2005)
11. Dafni, A.; Yaniv, Z.; Palevitch, D.: Ethnobotanical survey of medicinal plants in northern Israel. J Ethnopharmacol **10**, 295–310 (1984)
12. Yaniv, Z.; Dafni, A.; Palevitch, D.: Labiateae as medicinal plants in Israel. In: Aromatic plants: Basic and applied aspects. The Hague: Martinus Nijho, 265–269 (1982)
13. Güllüce, M., et al.: Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce sspserpyllifolia (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. J Sci Food Agric **84**, 735–741 (2004)
14. Shehab, N.G.; Abu-Gharbieh, E.: Constituents and biological activity of the essential oil and the aqueous extract of *Micromeria fruticosa* (L.) Druce subsp Serpyllifolia. Pak J Pharm Sci **25**(3), 687–92 (2012)
15. Salameh, N.; Shraim, N.; Jaradat, N.: Chemical composition and enzymatic screening of *Micromeria fruticosa* serpyllifolia volatile oils collected from three different regions of west bank, Palestine. BioMed. Research International, **2018**, Article ID 6536919 (2018)
16. Calín-Sánchez, Á., et al.: Effects of drying methods on the composition of thyme (*Thymus vulgaris* L.). Essent Oil Dry Technol **31**, 224–235 (2013)
17. Elwan, S., et al.: Effect of organ type, drying methods, and extraction time on yield and essential oil components of *Micromeria barbata* L. Int J Res Med Health Sci (IJRMHS). **4**(5), 43–46 (2014)
18. Venskutonis, P.R.: Effect of drying on the volatile constituents of thyme (Thymus vulgaris L.) and sage (Salvia officinalis L.). Food Chem. **59**(2), 219–227 (1997)
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute (2014)
20. Ben Jabeur, M.; Somai-Jemmali, L.; Hamada, W.: Thyme essential oil as an alternative mechanism: biofungicide-causing sensitivity of *Mycosphaerella graminicola*. J Appl Microbiol **122**, 932–939 (2017)
21. Zheljazkov, V.D., et al.: Chemical composition of the essential oil of the endemic species *Micromeria frivaldszkyana* (Degen) Velen. Molecules **24**, 440 (2019)
22. Thakur, S.A.; Nemade, S.N.; Sharanappa, A.: Solid state fermentation of overheated soybean meal (waste) for production of protease Using *Aspergillus Oryzae*. IJIRSET **4**(1), 18456–18461 (2015)
23. Miladi, H., et al.: Synergistic effect of eugenol, carvacrol, thymol, *p*-cymene and γ-terpinene on inhibition of drug resistance and biofilm formation of oral bacteria. Microb. Pathog. **112**, 156–163 (2017)
24. Dahham, S.S., et al.: The anticancer, antioxidant and antimicrobial Properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules **20**(7), 11808–11829 (2015)
25. Kamatou, G.P., et al.: Menthol: a simple monoterpene with remarkable biological properties. Phytochemistry **96**, 15–25 (2013)
26. Kumar, A.; Singh, S.P.; Chhokar, S.S.: Antimicrobial activity of the major isolates of mentha oil and derivatives of menthol. Anal Chem Lett **1**, 70–85 (2011)
27. Gong, H,; et al.: The specific effect of (R)-(+)-pulegone on growth and biofilm formation in multi-drug resistant Escherichia coli and molecular mechanisms underlying the expression of pgaABCD genes. Biomed Pharmacother, **134**, 111149 (2021)
28. Bassolé, I.H.; Juliani, H.R.: Essential oils in combination and their antimicrobial properties. Molecules **17**(4), 3989–4006 (2012)
29. Ultee, A., et al.: Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. J Food Prot **63**(5), 620–624 (2000)