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Chemical Composition of the Essential Oils of Three Thymus Taxa from Turkey with Antimicrobial and Antioxidant Activities

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Abstract: GC-MS analysis of the essential oils from aerial parts of *Thymus migricus* Klokov & Des.-Shost, *Thymus fallax* Fisch. & Mey. and *Thymus pubescens* Boiss. & Kotschy ex Celak var. *pubescens* resulted in the identification of 26, 35 and 53 constituents, respectively. The major components in the essential oil of *T. migricus* were found to be *α*-terpineol (30.6%), thymol (20.7%) and *α*-terpinyl acetate (14.9%) while in the essentiol oil of *T. fallax cis*-carveol (29.6%) and *α*-terpineol (10.8%). Carvacrol was a dominant compound with a percentage 66.1% of the essential oil of *T. pubescens* var. *pubescens.* The data obtained indicate that the essential oils of *Thymus* species generally exhibit some bacteriostatic activity. The antioxidant activity of the tested essential oils were found to be slightly lower than butylatedhydroxyanisole (BHA).

Keywords: *Thymus migricus; Thymus fallax; Thymus pubescens;* antimicrobial activity; antioxidant activity.

1. Introduction

Herbs/plants are the oldest friends of mankind. They have been employed in conventional medicine since ancient times, particularly due to their antimicrobial activity, and their medicinal properties have consequently been the object of frequent scientific study [1, 2]. According to the world health organization (WHO), about three-quarters of the world population rely upon traditional remedies (herbs/plants) for their health care [3].

In recent decades, the essential oils and various extracts of plant species have become popular as they have been the sources of natural products. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for effective compounds is becoming increasingly important [4]. Essential oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components [1]. Essential oils may be found in all of the aromatic plant species organs, serving important roles such as the protection of the plant against microorganisms, insects, and herbivorous animals or the attraction of insects responsible for the dispersion of pollens and seeds [5]. Essential oils of many plant species are known to have antimicrobial activity [6], and attempts to characterize their bioactive principles have gained

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momentum in many pharmaceutical and food-processing applications [7]. Several different types of spices have been evaluated as antimicrobial agents when applied against different pathogenic bacteria and fungi in vitro [8].

Among the aromatic plants belonging to the family Lamiaceae, the genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants [9], and thyme oils present high antimicrobial effect compared to the oils of other plants [10]. These antimicrobial properties are related to the chemical composition of the oils, which varies within the different species of the genus *Thymus* an even within the samples of the same species [11]. Thyme is stated to posses carminative, antispasmodic, antitussive, secretomotor, bactericidal, expectorant, astringent and anthelmintic properties [12].

The genus *Thymus* is represented in Turkey with 39 species (60 taxa), 20 of which endemic [13-15]. Members of this genus are called "kekik" in Turkish and most widely used as spices and in traditional folk medicine to treat infectious diseases and disorders [8]. Previous studies on the antimicrobial activity of the essential oils some *Thymus* spp. have shown activity against viruses [16], bacteria [17], and fungi [18, 19]. Although reports on the essential oils composition of different *Thymus* species are relatively common, investigations on their biological activities are still scarce.

In the present paper, we wish to report the chemical composition and antimicrobial and antioxidant activities of the essential oils produced by the aerial parts of *Thymus migricus* Klokov & Des.-Shost, *T. fallax* Fisch. & Mey., and *T. pubescens* Boiss. & Kotschy ex Celak var. *pubescens.*

2. Materials and Methods

2.1. Plant material

Samples of *Thymus* taxa were collected at flowering stage from East Anatolia (Turkey) in June 2008. Collection localities, dates, and essential oil yields are given in Table 1. Voucher specimens were deposited at the Herbarium of Inönü University (INU) in Malatya, Turkey.

Table 1. Plant materials used in this study

Species	Collection site	collection date	Oil yield[a](%)	Voucher[b]
T. migricus	Ağrı: Doğubeyazıt 1900-2000 m	28.06.2008	0.29	Yıldız 16818 & Arabacı
T. fallax	Bitlis: Tatvan, Nemrut Mountain, S. fece, 2100 m	29.06.2008	1.91	Yıldız 16822 & Arabacı
T. pubescens var. *pubescens*	Bitlis: Tatvan, Nemrut Mountain, around Kaldera, 2300 m	29.06.2008	0.08	Yıldız 16823 & Arabacı

[a] Calculated on moisture-free basis
[b] Collector number for Herbarium

2.2. Isolation of the Essential Oils

Air-dried aerial parts of plants were submitted to hydrodistillation for 3h using a Clevenger-type apparatus to produce the essential oils. The percentage yields (%) of the oils calculated on a moisture-free basis are shown in Table 1. Oils were dried over anhydrous sodium sulphate and, after filtration, stored at 4 ^{o}C until tested and analyzed.

2.3. GC and GC/MS analysis conditions

GC analysis was performed on an Agilent Technologies 6890N Network system gas chromatograph equipped with a FID and HP-Innowax column (60m x 0.25 mm i.d., 0.25 μm film thickness). Injector and detector temperature were set at 250 ^{o}C. The oven temperature was kept at 60 ^{o}C for 10 min and increased up to 220 ^{o}C at a rate of 4 ^{o}C min and then kept constant at 220 ^{o}C for 10 min and increased up to 240 ^{o}C at a rate of 1 ^{o}C and then kept constant at 240 ^{o}C for 10 min. Helium was the carrier gas, at a flow rate of 1.7 mL/min.

GC/MS analysis of the essential oil was performed under the same conditions with GC (column, oven, temperature, flow rate of the carrier gas) using an Agilent Technologies 6890N Network system gas chromatograph equipped with an Agilent Technologies 5973 inert Mass Selective Detector (Agilent G3180B Two-Ways Splitters with make up gas) in the electron impact mode (70eV). The mass range was between *m/z* 10 and 425.

2.4. Identification and quantification of essential oils constituents

The identification of volatile components was based on computer matching with the WILEY 7N, NIST05, and ADAMS libraries, as well as by comparison of the mass spectra and retention indices (RI) with those reported in the literature. Whenever possible, components were identified by comparison of their retention times, mass spectra and retention indices relative to n-alkanes with those of authentic standards available in author's laboratory. Percentage composition of the oil components were obtained from electronic integration using flame ionization detection (FID, 250 ^{o}C), without area normalization..

2.5. Antimicrobial Screening

The agar disc diffusion method was employed for the determination of antimicrobial screening of the essential oils [20]. Suspension of the tested microorganisms (10^8 CFU/mL) was spread on the solid media plates. Each test solutions were prepared in dimethyl sulphoxide (DMSO). Then filter paper discs (6 mm in diameter) were soaked with 20 µL of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h for bacteria and *Candida albicans*, *Campylobacter jejuni* incubated at 42 °C for 48 h. The diameter of the inhibition zones were measured in millimeters.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

For the determination of MIC micro-dilution broth susceptibility assay was used stock solutions of essential oils were prepared in (DMSO). Serial dilutions of essential oils were prepared in sterile distilled water in 96-well microtitter plates. Freshly grown bacterial suspension in double-strength Mueller–Hinton broth but *Listeria monocytogenes* in Buffered Listeria Enrichment Broth (Oxoid) and yeast suspension of *Candida albicans* in Saboraud Dextrose Broth were standardized to 10^8 CFU/mL (McFarland no. 0.5). Sterile distilled water served as growth control. 100 µL of each microbial suspension were then added to each well. The last row containing only the serial dilutions of antibacterial agent without microorganism was used as negative control. After incubation at 37 °C for 24 h (*Campylobacter jejuni* incubated at 42 °C for 48h) the first well without turbidity was determined as the minimal inhibitory concentration. Each test was performed in duplicate [20].

2.7. Fungal spore inhibition assay

In order to obtain conidia, the fungi were cultured on Czapex Dox Agar and Malt Extract Agar medium (Merck) in 9 cm petri dishes at 25 °C, for 7-10 days. Harvesting was carried out by suspending the conidia in a 1% (w/v) sodium chloride solution containing 5% (w/v) DMSO. The spore suspension was then filtered and transferred into tubes and stored at -20 °C [21]. The 1 mL spore suspension was taken, diluted in a loop drop until one spore could be captured. One loop drop from the spore suspension was applied onto the centre of the petri dish containing Czapex Dox Agar and Malt Extract Agar. 20 µL of each essential oil was applied onto sterile paper discs (6 mm in diameter) and placed in the petri dishes and incubated at 25 °C for 72 h. Spore germination during the incubation period was followed using a microscope (Olympus BX51) in 8 h intervals. The fungi *Aspergillus flavus, Aspergillus niger, Penicillum expansum, Alternaria alternate, Penicillium lanosum* were used for this assay and deposited in Balikesir University, Faculty of Science and Arts, Department of Biology (BUB), Balikesir, Turkey.

2.8. DPPH Radical Scavenging Assay

An essential oil solution (1 µg/mL) was prepared by dissolving the essential oil in methanol. Radical scavenging activity (RSA) of *Thymus* essential oils against stable 2,2-diphenyl-1–

picrylhydrazyl radical (DPPH) was determined by a slightly modified DPPH radical scavenging assay [22]. It is widely used reaction based on the ability of antioxidant molecule to donate hydrogen to DPPH; which consequently turns into an inactive form. The solution of DPPH was prepared daily. Briefly, 1mL of a 1mM solution of DPPH radical methanol was mixed with 3 mL of essential oil solution (final concentration of essential oil: 100-750 µg/mL), and left for 30 min (incubation period) in the dark at room temperature, the absorbance was read against a blank at 515 nm. This activity was given as % DPPH radical-scavenging calculated according to the equation:

$$\% \text{ DPPH radical-scavenging} = [(A_0 - A_S) / (A_0)] \times 100$$

where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_S is the absorbance of the tested sample. Test were carried out in triplicate and butylated hydroxyanisole (BHA) was used as positive control.

2.9. Statistical analysis

Means were compared using three- and one-way analysis of variance (ANOVA) and subsequently, means were separated using Tukey's Honestly Significant Difference (HSD) post hoc test. A statistical software program (SPSS, version 15.0 for Windows, SPSS Science, Chicago, IL) was used for data analysis. Results were considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1. Chemical composition of the essential oils

The results obtained by GC and GC/MS analysis of the essential oils of *T. migricus* (A), *T. fallax* (B), and *T. pubescens* var. *pubescens* (C) are shown in Table 2.

In the case of A, 26 compounds were identified representing the 80.4% of the total oil. *α*-terpineol (30.6%), thymol (20.7%), *α*-terpinyl acetate (14.9 %) and borneol (5.5%) were found to be the major constituents. Regarding the previously reported content of *T. migricus* essential oil [23], it is interesting to point out that there were important quantitative differences suggesting that the environmental factors and genotypes strongly influence its chemical composition.

Table 2. Essential oil composition (%) of *Thymus* taxa gathered from Turkey

Exp. RI[a]	Compound	A(%)	B(%)	C(%)	Exp. RI[a]	Compound	A(%)	B(%)	C(%)
1020	*α*-Pinene	nd	2.1	0.1	1390	Octen-3-yl acetate	nd	nd	0.2
1023	*α*-Thujene	nd	2.3	tr	1400	(*Z*)-3-hexen-1-ol	nd	tr	nd
1067	Camphene	nd	0.2	0.2	1450	3-Octanol	tr	0.1	tr
1118	*β*-Pinene	nd	0.2	0.2	1452	*trans*-Linaloloxide	nd	nd	0.1
1136	Sabinene	nd	0.1	0.3	1458	1-Isopropyl-4-methyl-1,3-cyclohexadiene	nd	nd	0.1
1172	*δ*-3-Carene	nd	0.1	nd	1460	1-Octen-3-ol	0.2	0.2	0.1
1192	Myrcene	nd	1.3	0.3	1475	*trans*-Sabinene hydrate	nd	1.0	0.8
1212	*α*-Terpinene	nd	1.0	0.1	1478	Menthone	0.7	nd	0.1
1237	Limonene	nd	0.2	0.3	1479	*cis*-Linaloloxide	nd	nd	0.1
1247	**1,8-Cineole**	0.9	1.4	**7.1**	1480	(Z)-3-hexenyl-2-methylbutrate	nd	tr	nd
1250	*β*-Phellandrene	nd	0.1	nd	1493	Octyl acetate	nd	nd	tr
1260	(*E*)-2-Hexenal	tr	nd	nd	1500	(*Z*)-3-hexenyl isovalarate	nd	tr	nd
1266	(*Z*)-*β*-Ocimene	nd	nd	0.6	1518	(*E*,*E*)-2,4-Heptadienal	nd	nd	tr
1275	***γ*-Terpinene**	nd	**4.6**	0.2	1536	Camphor	1.2	0.2	0.6
1286	(*E*)-β-Ocimene	0.2	**5.5**	**9.5**	1550	Benzaldehyde	nd	nd	tr
1290	3-Octanone	nd	1.5	nd	1559	Linalool	0.1	0.1	2.4
1296	***p*-Cymene**	0.2	**7.1**	0.2	1562	*p*-Menth-8-en-1-ol	0.2	nd	0.1
1301	Terpinolene	nd	0.1	0.1	1565	*cis*-Sabinene hydrate	nd	0.2	nd
1363	1-Octen-3-one	nd	nd	tr	1570	Linalyl acetate	nd	nd	1.7
1384	Neo-Allo-Ocimene	nd	nd	tr					

1600	Bornyl acetate	tr	nd	0.5
1604	*β*-Elemene	nd	nd	tr
1610	Thymol methyl ether	0.3	tr	nd
1614	6-Methyl-3,5-heptadien-2-one	nd	nd	tr
1616	Terpinen-4-ol	1.1	nd	nd
1618	***β*-Caryophyllene**	nd	1.5	**5.6**
1620	Carvacrol methyl ether	0.1	nd	nd
1632	Aromadendrene	nd	0.1	nd
1647	*p*-Mentha-6,8-dien-2-one	0.2	0.1	tr
1650	*trans*-*p*-Mentha-8-en-2-one	nd	0.2	nd
1653	Myrtenal	nd	nd	tr
1663	Allo-Aromadendrene	nd	nd	0.3
1665	Nonanol	nd	nd	tr
1669	*trans*-Pinocarveol	0.4	nd	nd
1674	Acetophenone	nd	0.1	nd
1685	(*E*)-*β*-Farnesene	nd	nd	0.2
1685	*γ*-Humulene	nd	tr	0.1
1688	*trans*-Verbenol	nd	nd	tr
1710	*γ*-Muurolene	nd	nd	tr
1714	***α*-Terpineol**	**30.6**	0.2	**10.8**
1715	***α*-Terpinyl acetate**	**14.9**	nd	nd
1726	**Borneol**	**5.5**	0.3	1.5
1728	Verbenone	0.2	nd	nd
1730	*α*-Amorphene	0.3	nd	nd
1732	Germacrene D	nd	nd	0.6
1740	Neryl acetate	nd	nd	0.1
1749	*β*-Bisabolene	nd	0.2	0.1
1750	Geranial	nd	nd	0.1
1755	Bicyclogermacrene	nd	0.1	2.4
1759	Carvone	0.2	tr	nd
1767	*cis*-Piperitol	0.1	nd	nd
1769	(*E*,*E*)-*α*-Farnesene	nd	nd	0.3
1775	Geranylacetate	nd	nd	0.4
1784	*δ*-Cadinene	0.2	nd	0.1
1803	*cis*-p-Menth-2-ene-1,8-diol	nd	nd	0.4
1805	Methyl salicylate	nd	tr	nd
1810	Myrtenol	0.2	nd	0.2
1842	*trans*-Carveol	0.5	nd	nd
1847	Geraniol	nd	nd	0.2
1860	*p*-Cymene-8-ol	0.7	tr	tr
1880	***cis*-Carveol**	nd	nd	**29.6**
1890	Ascaridole	nd	nd	tr
2008	Caryophyllene oxide	0.6	0.1	1.6
2049	**(*E*)-Nerolidol**	nd	nd	**7.5**
2069	Germacrene D-4β-ol	nd	nd	0.9
2102	Viridiflorol	nd	nd	0.1
2142	Spathulenol	nd	0.1	0.8
2183	T-Cadinol	nd	nd	0.2
2195	**Thymol**	**20.7**	0.3	tr
2204	T-Muurolol	nd	nd	0.1
2234	**Carvacrol**	0.4	**66.1**	**6.0**
2240	*trans*-*α*-Bergamotol	nd	nd	0.1
2253	*α*-Cadinol	nd	nd	0.3
	Total	**81.1**	**99.0**	**96.1**

[a]Retention indices relative to n-alkanes C_7-C_{29} based column HP-Innowax ; tr, trace (< 0.05 %); nd: not detected; A, *Thymus migricus*; B, *T. fallax*; C, *T. pubescens* var. *pubescens*

For example, *α*-terpineol was found to be the major constituent of *T. migricus* essential oil in our research (Table 2), it was assayed only in traces in previous report [23]. On the contrary, carvacrol, which was present at very low concentration (0.4%) in our sample, was detected as the main component in the previous report [23].

In the case of B, 35 compounds were identified representing the 99.0% of the total oil. Carvacrol (66.1%), *p*-cymene (7.1%), (*E*)-β-ocimene (5.5%) and γ-terpinene (4.6%) were found to be the major constituents. The essential oil of *T. fallax* from Turkey was characterized by a high content of carvacrol and low amount of thymol in the previous report [24]. In accordance with these findings, the essential oil of *T. fallax* contains mainly carvacrol (66.1%) and very low amount of thymol (0.3%). The chemical profile of our tested *T. fallax* essential oil was found to be good agreement with Tümen et al. [24] but, *T. fallax* oil from different localities in Iran was characterized by high content of thymol [25].

In the case of C, 53 compounds were identified representing the 96.1% of the total oil. *cis*-Carveol (29.6%), α-terpinol (10.8%), (*E*)-β-ocimene (9.5%), (*E*)-Nerolidol (7.5%),1,8-cineole (7.1%), β-caryophyllene (5.6%) and carvacrol (5.6%) were found to be the major constituents.

It was previously reported that oil of *T. vulgaris* L. contained thymol, *p*-cymene, γ-terpinene and carvacrol. *T. capitatus* Hoffmanns. & Link is very rich in carvacrol and *p*-cymene [26]; *T. migricus* and *T. fedtschenkoi* Ronniger var. *handelii* (Ronniger) Jalas in carvacrol, thymol and linalool [27]; *T. eriocalyx* (Ronniger) Jalas in thymol, linalool, γ-terpinene, 1,8-cineole, borneol and α-terpineol [28]. Bagamboula et al., investigated the essential oil of thyme, γ-terpinene (21.19%) and *p*-cymene (20.27%) [28]. Pinto et al. analyzed the composition of the essential oil of *T. pulegloides* from Portugal and the oil was characterized by high amounts of thymol (26.0%) and carvacrol (21.0%) and its biogenetic precurcors, γ-terpinene (8.8%) and *p*-cymene (7.8%) [29]. Kabouche et al. reported (60.8%) and *p*-cymene (10.3%) as the main components of the essential oils of *T. numidicus* [30]. The compositional data shows that carvacrol was the main compound in almost all samples. It is accepted that the terpenes, thymol, *p*-cymene and carvacrol are the major volatile components of thyme. Some studies have reported that thyme essential oil possesses a high level of the phenolic precursors, *p*-cymene and γ-terpinene [31]. Comparison between these results and the results of other reports showed differences, probably due to plant varieties or sites, as well as the time of harvesting.

3.2. Antimicrobial activity

The antimicrobial activity of *T. migricus, T. fallax* and *T. pubescens* var. *pubescens* essential oils assayed against human and food-borne microorganisms and their potency were qualitatively and quantitatively assayed by evaluating the presence of inhibition zones, zone diameter, and MIC values (Table 3 and 4). The *in vitro* results were classified as follows; if the extracts displayed a MIC of less than 100 μg mL^{-1}, the antibacterial activity was considered good; from 100 to 500 μg mL^{-1}, the antibacterial activity was considered moderate; from 500 to 1000 μg mL^{-1}, the antibacterial activity was considered weak; over 1000 μg mL^{-1} the extracts were considered inactive [32]. The antimicrobial activity of the essential oil of three *Thymus* expressed as MIC is given in Table 4. The essential oil of *T. migricus* presented moderate activity against *Campylobacter jejuni, Enterobacter aerogenes, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Proteus vulgaris* and *Candida albicans* with MIC at 250 μg mL^{-1} and weak activity against *Staphylococcus aureus* and *Serratia marcescens* with MIC at 500 μg mL^{-1}. The essential oil of *T. fallax* showed moderate activity against *Campylobacter jejuni, Enterobacter aerogenes, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus* and *Serratia marcescens* with MIC at 250 μg mL^{-1} and weak activity against *Candida albicans* with MIC at 500 μg mL^{-1}. The essential oil of *T. pubescens* var. *pubescens* displayed moderate activity against *Campylobacter jejuni, Enterobacter aerogenes, Escherichia coli, Listeria monocytogenes, Proteus vulgaris* and *Serratia marcescens* with MIC at 250 μg mL^{-1} and weak activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC at 500 μg mL^{-1}.

Table 3. Inhibition zones of essential oils according to agar disc diffusion method [mm].

Microorganisms	Stock solution			
	Diameter of inhibition zone (mm)			
	A	B	C	Control
Campylobacter jejuni ATCC 33291	8	9	8	25[C]
Enterobacter aerogenes NRRL 3567	9	10	9	22[C]
Escherichia coli ATCC 25292	9	9	9	22[C]
Listeria monocytogenes ATCC 7644	9	10	10	24[C]
Pseudomonas aeruginosa ATCC 27853	9	9	8	23[C]
Proteus vulgaris NRRL 123	10	9	9	24[C]
Staphylococcus aureus ATCC 6538	9	10	8	22[C]
Serratia marcescens Clinic isolate	8	9	9	24[C]
Candida albicans Clinic isolate	10	8	9	27[K]

A: *T. migricus*; B: *T. fallax*; C: *T. pubescens* var. *pubescens*
[C] : chloramphenicol [K] : ketoconazole

Table 4. Minimum inhibitory concentration [μg/mL] of essential oils

Microorganisms	A	B	C	Standard
Campylobacter jejuni ATCC 33291	250	250	250	-[C]
Enterobacter aerogenes NRRL 3567	250	250	250	-[C]
Escherichia coli ATCC 25292	250	250	250	-[C]
Listeria monocytogenes ATCC 7644	250	250	250	-[C]
Pseudomonas aeruginosa ATCC 27853	250	250	500	-[C]
Proteus vulgaris NRRL 123	250	250	250	-[C]
Staphylococcus aureus ATCC 6538	500	250	500	-[C]
Serratia marcescens Clinic isolate	500	250	250	-[C]
Candida albicans Clinic isolate	250	500	250	-[K]

A: *T. migricus*; B: *T. fallax*; C: *T. pubescens* var. *pubescens*
[C] : chloramphenicol; [K] : ketoconazole; - : no turbidity

In fact, phenolic compounds are capable of dissolving within the bacterial membrane and thus penetrating inside the cell, where they interact with cellular metabolic mechanisms [34,35]. The tested essential oils have been demonstrated to be efficient at inhibiting the growth of *A. niger*, *A. flavus, Penicillum expansum, P.lanosum and Alternaria alternata.* The essential oils are also active on fungi. However, treatment must be continued over a longer period. The results showed that *A. flavus* (23,43 %, 21,87 %, 32,80 %) and *Penicillum expansum* (21.42 %, 21.42 %, 25%) were more sensitive against the tested essential oils compare with other tested filamentous fungi (Table 5). Fundamental studies have revealed the antifungal activity of alcohols and sesquiterpene lactones.

Lawrence have established the composition of essential oils will depent on the plant species, the chemo-types and the climatic conditions, therefore their antimicrobial activities could vary [36]. This suggestion has been supported in the present study. Considering the large number of different groups of chemical compounds present in essentials oils, it is most likely that their antibacterial activity is not ascribable to one specific mechanism but that there are several targets in the cell [37]. An important special feature of essential oils and their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and proffering them more permeable [38]. As a rule, the essential oils possessing the strongest antibacterial properties against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol and

thymol [39]. Carvacrol is structurally very similar to thymol, having the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable [39]. The biological precursor of carvacrol, *p*-cymene is hydrophobic and induces swelling of the cytoplasmic membrane to a greater extent than does carvacrol [40].

Table 5. Antifungal activities of essential oils (% inhibition).

Microfungi	A	B	C	Ketoconazole
Aspergillus flavus	23,43	21,87	32,80	83,63
Aspergillus niger	11,66	10	16,6	40
Penicillum expansum	21,42	21,42	25	65
Penicillum lanosum	5,88	5,88	11,76	54
Alternaria alternata	8,92	14,28	12	82

A: *T. migricus*; B: *T. fallax*; C: *T. pubescens* var. *pubescens*

3.3. Antioxidant activity

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [41]. The scavenging ability of essential oils and positive control (BHA) are presented in Table 6. None of the tested *Thymus* species essential oils have found statistically significant activity ($p > 0.05$) against the DPPH.

The radical scavenging activity values of the essential oils *T. migricus, T. fallax* and *T. pubescens* var. *pubescens* were determined 13.29 ± 0.35%, 28.16 ± 0.24%, 10.24 ± 0.35% at 100 µg/mL concentration, respectively. Essential oil of *T. migricus* containing carvacrol (66.1%) among their main components showed moderate activities. Essential oils of *T. fallax*; *T. pubescens* var. *pubescens* were slightly active. Additionally, at the 750 µg/mL the essential oil concentrations of *T. migricus, T. fallax* and *T. pubescens* var. *pubescens* 45.36 ± 0.75%, 65.96 ± 0.12%, 42.29 ± 0.59% DPPH was scavenging. Nevertheless, it was 93.79 ± 0.75% in the presence of 100µg/mL BHA (Table 6).

The *in vitro* antioxidant activity of the essential oils of several *Thymus* species has been studied previously [42, 43]. The activities of the essential oils depend on several structural features of the molecules and attributed mainly to their content of phenolic components, particularly carvacrol and thymol [42], and the strong DPPH radical scavenging activity of those compound is well determined [44]. Also, on many others factors, such as concentration, temperature, light, type of substrate, physical state of the system, as well as on micro-components acting as pro-oxidants or synergists may influence the antioxidant activity [45].

Table 6. DPPH Radical-scavenging activity of essential oils.

Concentrations(µg/mL)	DPPH Scavenging ability (%, mean ± SD)*			
	A	B	C	BHA
100	13.29 ± 0.35 a	28.16 ± 0.24 a	10.24 ± 0.35 a	93.79 ± 0.75 a
125	16.91 ± 0.14 b	33.54 ± 0.62 b	13.87 ± 0.18 b	95.15 ± 0.33 a
250	22.94 ± 0.31 c	43.61 ± 0.63 c	18.53 ± 0.52 c	-
375	27.49 ± 0.33 d	49.00 ± 0.42 d	23.81 ± 0.49 d	-
500	34.30 ± 0.16 e	54.94 ± 0.33 e	29.03 ± 0.39 e	-
625	38.83 ± 0.13 f	59.63 ± 0.42 f	34.33 ± 0.49 f	-
750	45.36 ± 0.75 g	65.96 ± 0.12 g	42.29 ± 0.59 g	-

A: *T. migricus*; B: *T. fallax*; C: *T. pubescens* var. *pubescens*

*Each represents the mean of three replicates

Data in the columns (a-g) followed by the same letter are not significantly different ($p>0.05$).

BHA: Butylhydroxyanisole

SD: Standard Deviations

Concluding the results, the experiment led to new results in the field of the analytical characterization and antimicrobial activity and antioxidant capacity of *T. migricus*, *T. fallax* and *T. pubescens* var. *pubescens* essential oils.

In view of the observed inhibitory features of these essential oils, it is suggested that they could be used as preventatives against microfungal and bacterial contamination in many foods, instead of the common synthetic antimicrobial products. Also, the antioxidant activity of the tested essential oils was slightly lower than BHA. Thus, this study suggests the possibility of using the oils of these *Thymus* species as natural antioxidant and in the food industry, where they may be considered as natural preservatives to replace the synthetic preservatives of which consumers are increasingly distrustful. However, further research is needed to evaluate the effectiveness of *Thymus* species essential oils in food ecosystems to establish their utility as natural antimicrobial agents in food preservation and safety.

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