ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 5:3 (2011) 208-220

records of natural products

Chemical Composition, Radical Scavenging and Antimicrobial Activity of the Essential Oils of *Thymus boveii* and *Thymus hyemalis*

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(Received September 19, 2010; Revised October 5, 2011; Accepted December 10, 2011)

Abstract: This study was designed to examine the *in vitro* antimicrobial and antioxidant activities of the essential oil of *T. boveii* and *T. hyemalis*. According to the results of GC-EIMS analysis, essential oils were found rich in phenols and hydrocarbons. *p*-cymene, thymol and carvacrol were mainly found as the major compounds for the essential oils. Both plant species showed remarkable antioxidant activity in all test systems except chelating effect. In the case of antimicrobial activity, the oils showed remarkable growth inhibition against the tested microorganism except *K. pneumoniae*, *P. aeruginosa*, *L. monocytogenes*, *P. fluorescens*.

Keywords: *Thymus boveii*; *Thymus hyemalis*; antioxidant activity; GC-MS

1. Introduction

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes in various industries such as food, drugs and perfumery [1]. Researchers have been interested in biologically active compounds

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/15/2011EISSN:1307-6167

isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [2]. Plant products are also known to possess potential for food preservation [3-7].

Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity of food products, leading to their deterioration. Due to undesirable influences of oxidized lipids on the human organism, it seems to be essential to decrease contact with products of lipid oxidation in food [8]. In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. But, according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing such as, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have already been documented. For example, these substances can show carcinogenic effects in living organisms [9, 10]. From this point of view, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health [11].

The genus *Thymus* consists of approximately 215 species with *Thymus vulgaris*. This species is one of the most important and thoroughly investigated aromatic plants. Chemistry, processing and application of *Thymus* species were previously investigated. *Thymus* species as well as many other aromatic plants biosynthesize remarkable amount of volatile compound referred as the essential oil; therefore chemical classification of such plants is based on the main essential oil components. Among the major compounds available in the oil, thymol and carvacrol were reported to possess the highest antioxidant activity [12-15]. In addition, these compounds exhibit other biological activities, e.g. thymol is an antiseptic, while carvacrol possesses antifungal properties [16]. Non-volatile antioxidants such as flavonoids and vitamin E were also found in the extracts of *Thymus vulgaris* [17, 18]. Therefore, essential oils and/or non-volatile phytochemicals of thyme can be used as the natural preservative ingredients in food industries [19-24].

Like other *Thymus* species available in Turkish flora, *T. boveii* and *T. hyemalis* are called as ‘‘kekik”. According to our verbal communication with the local people from the collection area, herbal parts of this plant are used as tea and condiment. To the best of our knowledge, essential oil compositions of *T. hyemalis* have previously been reported [25-30], while no record is available for *T. boveii*. On the other hand, antimicrobial activity data is available for the both plant species [25, 31]. To the best of our literature search, no literature data is available for the antioxidant activities of them.

The aim of this study is to determine the antioxidant and antimicrobial activities of the essential oils of *T. boveii* and *T. hyemalis*. Data obtained from this study could be assumed as the first report for these species.

2. Materials and Methods

2.1 Isolation of the essential oil

The air-dried and ground aerial parts of the plants were submitted for 3 hours to water-distillation using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4 °C until tested and analyzed (yields 0.55% and 0.60% v/w, respectively).

2.2. Gas chromatography (GC) /EIMS analysis

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240 ^{o}C, respectively; oven temperature programmed from 60 to 240 ^{o}C at 3 ^{o}C/min; carrier gas helium at 1 ml/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against

commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data [32, 37]. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing.

2.3. Antioxidant activity

2.3.1. DPPH assay

Hydrogen atoms or electrons donation ability of the corresponding oils was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [38, 39]. Fifty µL of various concentrations of the oils in methanol was added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at 20°C the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in following way:

$$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated form the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used. Tests were carried out in triplicate. Values are presented as means ± S.D. of three parallel measurements.

2.3.2. β-Carotene-linoleic acid assay

In this assay antioxidant capacity is determined indirectly by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [17]. A stock solution of β–carotene-linoleic acid mixture was prepared as follows: 0.5 mg β–carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 µL linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL distilled water saturated with oxygen (30 min 100 mL/min.) was added with a vigorous shaking. 2.5 mL of this reaction mixture was dispersed to test tubes and 350 µL portions of the oils prepared at 2g/L concentrations were added and emulsion system was incubated up to 48 hours at room temperature. After this incubation period absorbance of the mixtures were measured at 490 nm. Antioxidative capacities of the oils were compared with those BHT and blank (contains EtOH instead of essential oil). Values are presented as means ± S.D. of three parallel measurements.

2.3.3. Reducing power

The reducing power was determined according to the method of Oyaizu [40]. Each of the samples (0.2-1.0 mg/mL) in methanol and water (2.5 mL) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricynide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. Finally, absorbance was measured at 700 nm against a blank.

2.3.4. Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. [41]. Briefly, 2 mL of various concentrations (0.25-1.00 mg/mL) of the samples in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorption readings at 562 nm were taken after 10 min against a blank sample consisting of a 2 mL extract solution with 2 mM $FeCl_2$ (0.05 mL) and water (0.2 mL) without ferrozine. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\textit{Metal chelating effect (\%)} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Where $A_{Control}$ is the absorbance of control (The control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{sample} is the absorbance of the test compound.

2.4. Antimicrobial activity

The essential oils were individually tested against a panel of microorganisms including *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Listeria monocytogenes* ATCC 19115, *Pseudomonas fluorescens* ATCC 49838, *Proteus mirabilis* ATCC 25933 and *Candida albicans* ATCC 90028. Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA). Yeasts were cultured overnight at 30°C in Sabouraud dextrose agar.

Disc-diffusion, microwell dilution and MIC agar dilution were performed following the methodology given in the previous study [42]. Ofloxacin (10 µg/disc), sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc) and netilmicin, (30 µg/disc) were used as positive reference standard antibiotic discs (Oxoid). Amphotericin B was also used as refererence antibiotic in micro well dilution (Sigma).

3. Results and Discussion

3.1. Chemical composition of the essential oils

The hydrodistillation of dried *T. boveii* and *T. hyemalis* buds gave reddish essential oils (yields 0.55% and 0.60% v/w, respectively). The identified compounds are shown in Table 1, according to their linear retention indices on a HP-5 capillary column.

The GC-EIMS analysis of the essential oil of *T. boveii* led to the identification of 60 different components, representing 97.41% of total oil constituents (Table 1). The essential oil contains a complex mixture consisting mainly phenols (53.02%), hydrocarbons (29.23%), alcohols (10.96%), ketones (2.24%), aldehydes (1.15%), epoxides (0.56%) and esters (0.25%). A portion (2.59%) of total composition was not identified. The major compounds of the essential oil were found as carvacrol (41.34%), *p*-cymene (19.80%), thymol (8.92%) and borneol (5.04%).

In the case of *T. hyemalis*, 70 compounds were identified, which represented about 98.09% of the total detected constituents. The major constituents of the oil were carvacrol (30.25%), thymol (18.32%), *p*-cymene (13.21%), γ-terpinene (6.35%) and verbenone (4.46%). The essential oil contains a complex mixture consisting mainly phenols (49.52%), hydrocarbons (31.92%), ketones (7.60%), alcohols (7.11%), esters (1.14%), aldehydes (0.55%) and epoxides (0.24%).

In this study, it was found that the percentage and compositions of essential oils obtained from the both plant species were significantly similar to each other, except some minor differences.

Table 1. Chemical composition of the essential oils of *T. boveii* and *T. hyemalis*

Compounds	LRI [a]	Relative Concentration (%)	
		T. boveii	***T. hyemalis***
Hydrocarbons			
Tricyclene	0927	-	0.04
α-Thujene	0930	0.10	1.02
α-Pinene	0939	1.33	0.60
Camphene	0954	1.72	0.65
Verbenene	0968	-	0.03
Sabinene	0975	0.16	0.10
β-Pinene	0979	0.31	0.05
Myrcene	0991	0.91	0.56
α-Phellandrene	1003	0.25	0.14
α-Terpinene	1017	1.29	2.41
p-cymene	1025	19.80	13.21
Limonene	1029	0.76	1.16
Δ-3-Carene	1031	-	0.11
(Z)-β-ocimene	1037	-	0.05
(E)-β-ocimene	1050	1.24	0.23
γ-Terpinene	1060	-	6.35
Terpinolene	1089	0.22	2.14
(E)-Caryophyllene	1419	0.65	2.59
Aromadendrene	1441	0.23	0.15
Alloaromadendrene	1460	-	0.02
Valencene	1496	0.26	0.21
δ-Cadinene	1523	-	0.10
Alcohols			
3-Methyl-2-buten-1-ol	0774	-	0.01
Hexanol	0871	0.23	0.05
1-Octen-3-ol	0979	-	0.06
3-Octanol	0991	0.11	-
2-Octanol	0995	0.15	0.01
Octanol	1068	0.03	-
Linalool	1097	2.10	0.80
Fenchol	1122	0.22	0.12
(E)-Pinocarveol	1139	0.27	0.08
(E)-Verbenol	1141	0.34	-
(Z)-Verbenol	1145	0.28	0.68
(E)-Sabinene hydrate	1154	0.42	0.83
(Z)-Sabinene hydrate	1156	0.11	0.06
Isoborneol	1162	-	0.01
Borneol	1169	5.04	2.62
Menthol	1172	0.01	-
Terpinen-4-ol	1177	0.61	0.45
p-Cymen-8-ol	1183	0.22	0.60
α-Terpineol	1189	0.43	0.25
Carveol	1229	0.09	0.05
Nerol	1230	0.07	0.01
Spathulenol	1578	0.23	0.42
Aldehydes			
(*E*)-2-Butenal	0738	-	0.01
Hexanal	0802	-	0.01
Furfural	0836	0.06	0.02
Heptanal	0902	0.91	0.01
(*E,E*)-2,4-Hexadienal	0910	0.05	-
(*E*)-2-Hexenal	1099	0.04	-

Nonanal	1101	0.01	0.12
Myrtenal	1196	-	0.05
Decanal	1202	-	0.23
Citronellal	1205	0.01	-
Neral	1238	0.07	0.08
Geranial	1267	-	0.03
Ketones			
3-Heptanone	0892	0.04	0.01
3-Octanone	0984	-	0.01
β-Thujone	1114	0.02	0.13
Camphor	1146	0.41	2.52
Menthone	1153	0.17	0.30
Dihydrocarvone	1193	0.26	0.16
Verbenone	1205	1.34	4.46
Thymoquinone	1252	-	0.01
Esters			
Ethyl acetate	0806	-	0.01
Benzyl acetate	1162	-	0.82
Ethyl caprilate	1241	-	0.01
Linallyl acetate	1257	0.05	-
Isobornyl acetate	1286	0.01	-
Bornyl acetate	1289	-	0.15
Terpinyl acetate	1349	0.01	-
Citronellyl acetate	1353	0.06	0.11
Neryl acetate	1362	0.12	0.04
Phenols			
Carvacrol methyl ether	1245	0.96	0.89
Thymol	1290	8.92	18.32
Carvacrol	1299	41.34	30.25
Eugenol	1359	1.24	0.05
Isoeugenol	1451	0.56	0.01
Epoxides			
(*Z*)-Limonene oxide	1137	0.01	0.07
(E)-Limonene oxide	1142	0.19	-
Caryophyllene oxide	1583	0.36	0.17
	Total	**97.41**	**98.09**

Compound Classes	***T. boveii* (%)**	***T. hyemalis* (%)**
Hydrocarbons	29.23	31.92
Alcohols	10.96	7.11
Aldehydes	1.15	0.55
Ketones	2.24	7.60
Esters	0.25	1.14
Phenols	53.02	49.52
Epoxides	0.56	0.24

[a] LRI, linear retention indices (HP-5 column); [b] tr, trace (60.1%)

As far as our literature survey could as certain, no report is available for the essential oil composition of *T. boveii*. Therefore, this study could be assumed as the first report on this topic. On the other hand, numerous reports are available in the literature on the effect of seasonal variations and environment on *T. hyemalis* essential oil [26, 27, 29, 43]. In these reports, mainly three different chemotypes of *T. hyemalis* have been revealed namely thymol, thymol/linalool and carvacrol. The thymol chemotype is widespread and is found in most of the vegetal formations where *T. hyemalis* is predominant and does not interact with other species [30]. According to the information provided in these articles, *T. hyemalis* essential oil presented here can be considered in carvacrol chemotype.

3.2. Antioxidant activity

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts [44]. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. The methods chosen are the most commonly used for the determination of antioxidant activities of plant extracts and/or essential oils.

The reduction ability of DPPH radicals' formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [45].

The scavenging ability of the essential oils showed a concentration-dependent activity profile (Table 2). The strongest free radical scavenging activity was exhibited by *T. boveii* essential oil (82.75% ± 1.15 at 0.50 mg/mL). Free radical scavenging capacity of *T. hyemalis* was determined as 73.48% ± 0.56 at the same concentration. Free radical scavenging potentials of the synthetic antioxidant BHT, α-tocopherol and quercetin were determined as 30.83% ± 0.32, 52.92% ± 2.21 and 95.88% ± 0.51, respectively at 0.02 mg/mL concentration.

As clearly indicated by Lucarini et al. [46] radical-scavenging capacity is directly related to the hydrogen atom donating ability of a compound and not correlated to the redox potentials alone, as observed by this researcher when studying the antioxidant capacity of phenothiazine and other related compounds.

In β-carotene-linoleic acid system, β-carotene undergoes a rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β-carotene destruction by "neutralizing" the linoleate free radical and any other free radicals formed within the system [47]. Table 2 depicts the inhibition of β-carotene bleaching by the essential oils of *T. boveii* and *T. hyemalis*.

As can be seen from Table 2, the most active sample was the essential oil of *T. boveii*. At 2.0 mg/mL concentration, antioxidant activity of the oil was measured as 97.51 ± 0.57%. At the same concentration, the essential oil of *T. hyemalis* also exhibited excellent activity value (89.33% ± 1.12). In this test system, oxidation of linoleic acid by the synthetic antioxidant BHT, α-tocopherol and quercetin were determined as 96.04% ± 0.58, 96.43% ± 0.32 and 98.39% ± 0.58, respectively at 0.40 mg/mL concentration.

The reductive potential measures the ability of a sample to act as electron donor and, therefore, reacts with free radicals converting them to more stable products and thereby terminates radical chain reactions.

Reducing power of the samples is also presented in Table 2. As can be seen from the table, reducing power of the essential oils of the both plant species found too close to the each other at 1.0 mg/mL concentration (0.565 nm ± 0.012 and 0.548 nm ± 0.010, respectively). It is extremely important to point out that, reductive potentials of the extracts and/or essential oil are strictly related with the polarities of their phytochemicals. The essential oil, which contains the non-polar secondary metabolits (terpenoids), remains almost inactive. Reducing powers of BHT, a-tocopherol and ascorbic acid were measured as 0.800 nm ± 0.004, 0.512 nm ± 0.007 and 1.184 nm ± 0.083, respectively at 0.2 mg/mL concentration.

The reductive potential, measured by the absorbance at 700 nm, may be due to the di- and monohydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities as described by Shimada et al. [48]. Nevertheless our results seem to reveal the existence of some minor components, other than carvacrol, the main phenol compound present in these oils, responsible for the significant reductive ability of the oils.

Table 2. Antioxidant activity of the essential oils of *T. boveii* and *T. hyemalis* [a]

Test systems	Concentrations (mg ml^{-1})	Samples						
		T. boveii	***T. hyemalis***	**BHT**	**α-tocopherol**	**Quercetin**	**Ascorbic acid**	**EDTA**
DPPH (%)	0.02	-	-	30.83 ± 0.32	52.92 ± 2.21	95.88 ± 0.51	-	-
	0.10	35.71 ± 1.21	28.64 ± 1.36	-	-	-	-	-
	0.20	57.26 ± 1.68	41.34 ± 1.40	-	-	-	-	-
	0.50	82.75 ± 1.15	73.48 ± 0.56	-	-	-	-	-
β-Carotene / Linoleic acid (%)	0.4	89.46 ± 1.63	79.14 ± 1.33	96.04 ± 0.58	96.43 ± 0.32	98.39 ± 0.58	-	-
	1.0	95.42 ± 1.32	84.63 ± 1.26	-	-	-	-	-
	2.0	97.51 ± 0.57	89.33 ± 1.12	-	-	-	-	-
Reducing Power (absorbance at 700 nm)	0.2	0.182 ± 0.001	0.134 ± 0.004	0.800 ± 0.004	0.512 ± 0.007	-	1.184 ± 0.083	-
	0.4	0.243 ± 0.005	0.229 ± 0.007	-	-	-	-	-
	1.0	0.565 ± 0.012	0.548 ± 0.010	-	-	-	-	-
Chelating Effect (%)	0.25	N.A.	N.A.	-	-	-	-	99.42 ± 0.07
	0.50	N.A.	N.A.	-	-	-	-	-
	1.0	N.A.	N.A.	-	-	-	-	-

[a] Values expressed are means ± S.D. of three parallel measurements
N.A.: Not Active

Table 3. Antimicrobial activity of the essential oils of *T. boveii* and *T. hyemalis*

Microorganisms	Samples					
	T. boveii		***T. hyemalis***		**Antibiotics** [a]	
	DD [b]	MIC [c]	DD	MIC	DD	MIC
B. cereus	22.00 ± 0.11	15.62	20.50 ± 0.46	31.25	28.00 (OFX)	62.50
B. subtilis	19.50 ± 0.26	31.25	17.30 ± 0.64	31.25	28.00 (OFX)	125.00
E. aerogenes	16.50 ± 0.11	62.50	-	-	20.00 (NET)	31.25
E. faecalis	18.74 ± 0.42	31.25	14.20 ± 0.23	62.50	18.00 (SCF)	31.25
E. coli	13.50 ± 0.26	31.25	11.60 ± 0.30	125.00	12.00 (OFX)	125.00
K. pneumonia	14.40 ± 0.73	62.50	-	-	12.00 (OFX)	125.00
P. aeruginosa	-	-	-	-	22.00 (NET)	15.62
S. aureus	17.40 ± 0.26	62.50	15.00 ± 0.74	62.50	22.00 (SCF)	31.25
S. epidermidis	12.65 ± 0.35	62.50	9.50 ± 0.23	125.00	12.00 (SCF)	15.62
L. monocytogenes	10.00 ± 0.65	125.00	-	-	12.00 (OFX)	125.00
P. fluorescens	10.50 ± 0.46	125.00	-	-	18.00 (NET)	125.00
P. mirabilis	8.00 ± 0.23	250.00	8.00 ± 0.46	250.00	12.00 (OFX)	125.00
C. albicans	18.50 ± 0.26	31.25	16.40 ± 0.26	62.50	28.00 (Amp B)	31.25

[a] OFX: Ofloxacin (10 µg/disc); SCF: sulbactam (30 µg)+cefoperazona (75 µg) (105 µg/disc) and NET: Netilmicin, (30 µg/disc) were used as positive reference standards antibiotic discs (Oxoid); AmpB: Amphotericin B was used as refererence antibiotic in micro well dilution (Sigma).
[b] DD: Disc Diffusion, Inhibition zone in diameter (mm) around the discs impregnated with 300 µg/disc of methanol extract. [c] MIC: Minimal Inhibitory concentrations as (µg/ml)

Transition metal ions can stimulate lipid peroxidation by two mechanisms, namely by participating in the generation of initiating species and by accelerating peroxidation decomposing lipid hydroperoxides into other components which are able to abstract hydrogen, perpetuating the chain of reaction of lipid peroxidation [49].

Data revealed from the chelating effect experiments are presented in Table 2. In this system; essential oils of the both plant species did not show metal chelating effect. Metal chelating potential of EDTA was measured as 99.42% ± 0.07 % at 0.25 mg/mL concentration.

The differences found with the different methodologies can be to a certain extent explained by the diverse relative amounts of minor compounds in the oils but that can have a major impact in the final oil antioxidant effect. Further work is needed to fully understand the variables that can affect the evaluation of the antioxidant capacity by different methodologies.

In general, essential oil of *T. boveii* exhibited slightly greater antioxidant activity than that of *T. hyemalis*. As far as our literature survey could as certain, antioxidant activity of *T. boveii* and *T. hyemalis* have not previously been reported. From this point of view, the results presented in Table 2 could be assumed as the first data on these plant species.

3.3. Antimicrobial activity

As far as the in vitro antimicrobial activity results are concerned, the essential oils in general possessed extremely strong activity potential (Table 3).

In the presence of *T. boveii* essential oil the strongest activity was observed against *B. cereus* with an MIC at 15.62 mg/mL followed by *B. subtilis*, *E. faecalis* and *C. albicans,* with MIC at 31.25 mg/mL. The weakest activity was observed against *P. mirabilis* (MIC, 250.00 mg/mL). The oil did not show activity against *P. aeruginosa.*

In general, *T. hyemalis* essential oil showed weaker antimicrobial activity than that of *T. boveii*. In the presence of this sample, no activity was observed against *E. aerogenes, K. pneumonia, P. aeruginosa, L. monocytogenes* and *P. fluorescens*. The most sensitive microorganisms were determined as *B. cereus* and *B. subtilis* with a MIC value of 31.25 mg/mL. This activity was followed by *E. faecalis, S. aureus, C. albicans* (62.50 mg/mL).

The growth inhibitions of test microorganisms were also evaluated by using the main constituents of the essential oils (carvacrol, thymol and *p*-cymene) individually in broth microdilution method (Table 4). The lowest MIC value was found in the presence of carvacrol against *B. cereus* and *C. albicans* (0.24 mg/mL), followed by E. coli (0.48 mg/mL), *E. aerogenes* and *P. mirabilis* (1.95 mg/mL). *P. aeruginosa* was the most resistant microorganism with a MIC at 7.81 mg/mL). As can be seen from the table, *p*-cymene, precursor of carvacrol, could not be able to inhibit the growth of microorganisms in general.

The antimicrobial properties of the oils are suspected to be associated with the carvacrol content, which has been tested previously and was found to have a significant antibiotic activity [50]. Also, synergism between carvacrol and its precursor *p*-cymene has been noted. Ultee et al. [51] showed that *p*-cymene is a very weak antibacterial, and swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism *p*-cymene probably enables carvacrol to be more easily transported into the cell so that a synergistic effect is achieved when the two are used together.

4. Conclusion

When considering from an applicability point of view, this study shows that the essential oils of *T. boveii* and *T. hyemalis* attained the remarkable activity to prevent lipid oxidation. It is thus noteworthy to point out the interest in investigating the plants showing the highest biological activities. In future, these plants may be under the designation of protected origin, due to their bioactive constituents. On the other hand, both plant species also showed significant antimicrobial activity. It is very interesting that, the activity especially focused towards food poisoning

microorganisms. These studies point out the importance of comparing and exploring their use either in food industries or for medical purposes.

Table 4. The minimum inhibitory concentrations (MIC) of commercially available major components

Microorganisms	Commercially available essential oil components		
	Thymol	*Carvacrol*	*p-cymene*
S. aureus	1.95	0.48	≥ 250.00
B. cereus	0.97	0.24	250.00
E. aerogenes	0.97	1.95	≥ 250.00
E. coli	1.95	0.48	≥ 250.00
K. pneumoniae	1.95	3.90	≥ 250.00
P. mirabilis	1.95	1.95	≥ 250.00
P. aeruginosa	15.62	7.81	≥ 250.00
C. albicans	0.97	0.24	15.62

Acknowledgements

Financial support from the Research Council of Cumhuriyet University, Sivas-Turkey (Project No: F-256) is gratefully acknowledged.

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