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Chemical Composition and Biological Activities of Essential Oil of *Thymus haussknechtii* Velen. from Turkey

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The genus *Thymus* L. is represented by 40 species (42 taxa) in Turkey, of which 18 are endemic. *Thymus haussknechtii* Velen. is endemic species of the genus. In this study, the essential oil of *Thymus haussknechtii,* obtained by hydrodistillation from the aerial parts, were analyzed by GC/MS. The main constituents of the oils are identified and antioxidant and antibacterial bioassays were determined. Isoborneol (9.06 %), linalool (8.44 %), α-pinene (6.45 %), γ-terpinene (6.08 %), camphene (5.12 %), caryophylleneoxide (5.11 %) are established as the major components of the essential oil. The antibacterial activity was evaluated against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. Both tested bacteria were inhibited by the essential oil. The essential oil of the species was subjected to a screening for their possible antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS. A remarkable antioxidant activity was observed from the essential oil according to the comparison with the antioxidative potentials of the standard compound used in this study. The findings of the analyzed taxa are compared with the results of previous studies.

Keywords: Endemic, Essential oil composition, DPPH, GC/MS, Thyme.

INTRODUCTION

Thymus L. is an aromatic, ornamental and medicinal genus of Lamiaceae, including about 220 species, distributed in Europe, Mediterranean region and Macaronesia, Asia, North Africa and mountains of Ethiopia [1]. According to recent studies of the Turkish *Thymus*, this genus is represented in Turkey by 40 species (42 taxa), of which 18 are endemic [2]. Thyme is an aromatic plant used for medicine, spice, pharmaceutical and cosmetic industries as a flavouring [3,4].

Thymus haussknechtii Velen is endemic species of the genus. This study includes the analyses of essential oil of *T. haussknechtii,* obtained by hydrodistillation from the aerial parts, by GC/MS. The main constituents of the oils are identified and antioxidant and antibacterial bioassays were determined.

EXPERIMENTAL

Isolation of the oils: Aerial parts of *Thymus haussknechtii* were collected in the blooming stage from Elazig province, in Turkey. The specimens were dried by air dried method. The aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus. Voucher specimen (No: Arabaci 2871) of species is deposited in the herbarium of the Faculty of Pharmacy, Inonu University, Malatya, Turkey.

Essential oil analysis: The oils were analyzed by GC/MS, using an Agilent Technologies 6890N Network system gas chromatograph equipped with a FID and HPInnowax column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Injector and detector were heated at 250 °C. The oven temperature was linearly programmed from 60 to 250 °C (5 °C/min) and then kept constant at 250 °C for 20 min. Helium was used as the carrier gas, at a flow rate of 1.7 mL/min. GC/MS analyses were carried out under the same conditions (column, oven, temperature, flow rate of the carrier gas) with GC by Agilent Technologies 6890N Network system gas chromatograph equipped with an Agilent Technologies 5973 inert mass selective detector (Agilent G3180B Two-Ways Splitters with Makeup gas) in the electron impact mode (70 eV). The mass range was between *m/z* 10 and 550. The column, temperature programme and injection were performed as described above. Injection was carried out automatic mode. Library search was carried out using Wiley7N, Nist02, Nist05 and Adams. Relative percentages were calculated. For quantification purposes area

percent reports obtained by FID were used. Chemical composition of the essential oils and their relative percentages are listed in Table-1.

TABLE-1
CHEMICAL COMPOSITION, RETENTION INDICES (RI), PERCENTAGE (%) COMPOSITION OF THE ESSENTIAL OIL OF *Thymus haussknechtii*

Compound	RI	Composition (%)
α-Pinene	**1045**	**6.45**
Camphene	**1071**	**5.12**
β-Pinene	1103	0.83
Sabinene	1115	0.95
Myrcene	1153	0.65
α-Terpinene	1168	3.92
Limonene	1185	1.35
Cineole	1194	4.47
Z-β-Ocimene	1213	0.35
γ-Terpinene	**1226**	**6.08**
δ-3-Carene	1230	2.48
Terpinolene	1250	1.44
Terpineol	1367	4.55
Linaloxide	1370	0.29
Chrysanthenone	1390	0.03
Camphor	1398	4.07
Linalool	**1412**	**8.44**
cis-β-Terpineol	1420	1.95
Isopinocarveol	1424	0.57
Norborneol	1432	0.77
Neodihydrocarveol	1453	0.16
Isoborneol	**1494**	**9.06**
α-Phellandren-8-ol	1502	0.70
Germacrene D	1652	2.00
2,6-Octadienal	1748	0.30
2-Cyclohexen-1-ol	1806	1.03
Nerol	1849	0.28
Caryophyllene oxide	**1885**	**5.11**
Nerolidol	1965	4.76
α-Elemol	2009	4.33
p-Cymen-7-ol	2025	0.17
Spathulenol	2046	2.98
Guaiacol	2080	0.18
Epi-Eudesmol	2114	1.06
Decanoicacid	2130	0.08
(4S,5R)-5-Hydroxycaryophyllene-4,12-epoxide	2186	0.07
Caryophyllenol II	2193	0.68
Phthalic acid	2322	0.08
n-Hexadecanoic acid	2447	0.30

Antibacterial activity: The bacteria *Staphylococcus aureus* ATCC 29213 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative) were used. The strains were obtained from Department of Biology, Faculty of Science and Arts, Inonu University, Malatya, Turkey. The broth microdilution method, quantitative reference method routinely used in clinical laboratories, was used for determine the minimum inhibitory concentrations (MICs) of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213). This method carried out with susceptibility panel in 96-well microtiter plates that including various concentration of antimicrobial agents. The numbers of standardized bacteria were inoculated into the wells of 96-well microtiter and incubated at 37 °C for 24 h. The MIC value was observed as the lowest concentration where no viability was observed in the wells of 96-microwell plates after incubation. Optical density (OD) was determined at 600 nm using a microtiter plate reader [5] (Table-2).

TABLE-2
RESULT OF THE BROTH MICRODILUTION ASSAY [MIC (mg/mL)]

Sample code antibacterial parameter MIC (mg/mL)	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 29213)
Thymus haussknechtii	7.8625	7.8625

Antioxidant activity: The DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay was conducted by changing the method used by Hwang *et al*. [6] DPPH (1,1-diphenyl-2-picrylhydrazyl) was dissolved in methanol and then diluted with the same solvent to achieve an absorbance of 0.70 ± 0.0 at 517 nm. The mixture of DPPH, test oil and methanol was kept in dark 0.5 h at room temperature. The absorbance was read against a blank at 517 nm to compare with the standard (Trolox) by measuring their absorbance. Radical scavenging properties of essential oil was given as mg Trolox equivalent/mL essential oil (Table-3). The ABTS radical scavenging activities followed that of Re *et al*. [7]. ABTS radical was obtained from potassium peroxidisulfate (6.6 mg) and ABTS (30 mg) in 7.8 mL aqueous solution that standing for 12-16 h at room temperature. The absorbance of the resulting coloured solution 0.70 ± 0.0 at 734 nm was diluted to obtain the necessary ABTS solution to compare with the standard (Trolox) by measuring their absorbance. The results were determined as mg Trolox equivalent/mL essential oil (Table-3).

TABLE-3
RADICAL SCAVENGING PROPERTIES OF ESSENTIAL OIL (EO) OF *T. haussknechtii* (mg TROLOX EQUIVALENT/mL EO)

Sample name	DPPH	ABTS
Thymus haussknechtii	0.97 ± 0.037	12.76 ± 1.65

RESULTS AND DISCUSSION

The compounds of the essential oil of *T. haussknechtii* are given in Table-1. The amount of oil calculated per weight of the dried plant material (v/w) was 0.6 %. The numbers of the identified compounds are 39 and representing 88.09 % of the oil. From our results it can be seen that isoborneol (9.06 %), linalool (8.44 %), α-pinene (6.45 %), γ-terpinene (6.08 %), camphene (5.12 %) and caryophyllene oxide (5.11 %) are the major components of the oil. The results are compared with the other chemotypes given in previous studies [8-10]. Comparing the previous data with the chemical composition of the oil, it becomes evident that 1,8-cineole, linalool, caryophyllene oxide, camphor, α-pinene, γ-terpinene, borneol and isoborneol are the major common components of the *T. haussknechtii* that grown from different localities (Table-4).

The essential oil of species are tested against Gram-positive *Staphylococcus aureus* ATCC 29213 and Gram-negative *Escherichia coli* ATCC 25292 bacteria using broth microdilution assay. The essential oil is active Gram-positive and Gram-negative bacteria. The antimicrobial activities of

TABLE-4
COMPARISON OF CHEMOTYPE OF ESSENTIAL OIL OF *T. haussknechtii*

Locality	Major components		Ref.
	Compound	Composition (%)	
Elazig, 1 km north of Kömürhan, dam lake side, 650 m	Isoborneol	9.06	Present study
	Linalool	8.44	
	α-Pinene	6.45	
	γ-Terpinene	6.08	
Erzincan, Kemaliye-Arapkir way, Firat Valley 900-1000 m	1,8-Cineole	23.60	[10]
	trans-Verbenol	6.60	
	Camphor	6.12	
	Caryophyllene oxide	6.00	
Elazig	Linalool	19.90	[8]
	Borneol	10.35	
	Caryophyllene oxide	7.14	
	1,8-Cineole	6.11	
Elazig, Harput, Ankuzubaba mountain, 1200 m	1,8-Cineole	21.50	[9]
	Linalool	6.40	
	Borneol	4.40	
	Camphor	6.80	

essential oils from the MIC values are 7.8 mg/mL for each bacteria (Table-2). Antimicrobial activity of the 1,8-cineole, *trans*-verbenol, camphor and caryophyllene oxide have been previously reported for *T. haussknechtii* [10].

Radical scavenging properties of essential oil (EO) obtained from *T. haussknechtii* (mg Trolox equivalent/mL EO) is determined as 0.97 ± 0.037 for DPPH and 12.76 ± 1.65 for ABTS (Table-3). The % DPPH radical scavenging activity values of the essential oil *T. haussknechtii* was given as percent inhibition and determined as 6.90 ± 0.15 % at 100 µg/mL concentration and 35.11 ± 0.22 % at 1000 µg/mL in previous study [10].

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