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Chemical Composition, Antioxidant Capacity, Acetyl- and Butyrylcholinesterase Inhibitory Activities of the Essential Oil of *Thymus haussknechtii* Velen.

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Abstract: The chemical composition of the essential oil from the aerial parts of *Thymus haussknechtii* Velen. was analyzed by using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The major component of the essential oil was thymol (52.2%). Total phenolic content of the essential oil was determined as 132.9 µg gallic acid equivalent. The antioxidant capacity was evaluated by DPPH free radical, superoxide anion radical and hydrogen peroxide scavenging activities along with ferrous ion-chelating power test, ABTS radical cation decolorization assay and ferric thiocyanate methods. In addition to antioxidant activity, anticholinesterase activity of the essential oil was also evaluated. It exhibited inhibitory activities on AChE and BuChE which play an important role in Alzheimer's disease, along with significant antioxidant activity.

Keywords: *Thymus haussknechtii*; essential oil; antioxidant activity; acetylcholinesterase; butyrylcholinesterase.

1. Plant Source

The genus *Thymus* (Lamiaceae) is represented by 43 species in Turkey [1-4] and the biological activity studies showed that the essential oil of some *Thymus* species indicated antioxidant, antimicrobial, insecticidal [5-8], antiprobiotic [9], antibacterial [10-11] activities.

The aerial parts of *Thymus haussknechtii* Velen. were collected from Mount Kop in Semptember 2013 (2030 m) and identified by Mehmet Önal from Regional Directorate of Forestry, Türkiye. The

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voucher specimen (AUEF 1001) has been deposited in the herbarium of the Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye.

2. Previous Studies

A few study have been carried out on the chemical composition of the essential oil of *T. haussknechtii* which were collected from different places of Turkey and exhibited different chemical profiling [7, 12-14]. The main constituents of the oil of *T. haussknechtii* collected from Erzincan were reported as 1,8-cineole (23.6%), *trans*-verbenol (6.6%), camphor (6.12%) and caryophyllene oxide (6%). Furthermore, its antioxidant activity was determined by means of the DPPH radical-scavenging method and at 1000 µg/mL concentration of the essential oil 35.11 ± 0.22 % DPPH was scavenged [7]. The essential oil of *T. haussknechtii* collected from Elazığ province was found to possess linalool (19.9%) and borneol (10.3%) as major components [13]. Finally, 1,8-cineole (23.6%) was designated as the main constituent in the oil of *T. haussknechtii* collected from Elazığ Harput-Ankuzubaba Mountain [14].

3. Present Study

Chemical composition of the essential oil: Hydrodistillation of the aerial parts of *T. haussknechtii* collected from Kop Mount, gave light yellow oil with 1.34% (w/w) yield. The essential oil composition was analyzed by GC-FID and GC-MS. Forty nine components constituting 99.6% of the essential oil were identified. Thymol was the main constituent of *T. haussknechtii* essential oil with 52.2% of the total oil composition. All components of the essential oil identified by GC and GC–MS analyses were listed in Table 1 along with their relative retention indices and their percentage composition.

GC analysis of the essential oil was carried out on Agilent 5975 GC-MSD system and Agilent 6890N GC system with flame ionization detector (FID) and a HP-Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness). Helium was used as a carrier gas at 0.8 mL/min. The injector and detector temperatures were 250°C and 300°C, respectively. GC oven temperature was at 60°C for 10 min, then programmed to 220°C at a rate of 4°C/min and kept in this temperature for 10 min and finally programmed to 240°C at a rate of 1°C/min with a final hold time of 80 min. The split ratio was 40:1. Mass spectrums were taken at 70 eV and mass range was from *m/z* 35 to 450. In order to obtain same elution order with GC-MS, simultaneous injection was done by using the same column and appropriate operational conditions.

In consideration of previous studies, the major constituents of *T. haussknechtii* oils from different localities were found to be 1,8-cineole, *trans*-verbenol, camphor, caryophyllene oxide, linalool and borneol in the range of 6-23.6%. However, the main component of our essential oil was thymol with a high amount (52.2%). These results indicates that geographical location, collection season and environmental factors may be the reason of these variations in the essential oil composition with important quantitative differences [6-7,12-14].

Total phenolic content of the essential oil: Total phenolic content of the essential oil was determined according to the Folin–Ciocalteu's method [15] using gallic acid as a standard phenolic compound. Total phenolic content of the essential oil was calculated by using the equation that was obtained from the standart gallic acid graph (Figure S1). 132.9 ± 0.2 µg gallic acid equivalent of phenolic compounds were detected in 1 mg of the essential oil.

Antioxidant activity tests: Antioxidant activity was evaluated on six tests: DPPH free radical scavenging activity [16], ferrous ion-chelating power test [17], ABTS radical cation decolorization assay [18], superoxide anion radical scavenging activity [19], total antioxidant activity by ferric thiocyanate method [20], hydrogen peroxide scavenging activity [21], which were carried out as described in the literatures. The scavenging effect of the essential oil of *T. haussknechtii*, thymol as the major component of the essential oil and reference compound trolox on DPPH, ABTS, H_2O_2 and superoxide radical were evaluated.

Table 1. Chemical composition of the essential oil of *T. haussknecthii.*

Compound	RRI[a]	%[b]
Tricyclene	1014	t[c]
α-Pinene	1032	0.7
α-Thujene	1035	0.7
Camphene	1076	0.5
β-Pinene	1118	0.3
Sabinene	1132	0.1
δ-3-Carene	1159	t
Myrcene	1174	0.8
α-Terpinene	1188	0.9
Limonene	1203	0.3
1,8-Cineole	1213	4.6
γ-Terpinene	1255	6.6
3-Octanone	1266	0.1
p-Cymene	1280	16.6
Terpinolene	1290	0.2
3-Octanol	1393	0.1
α,*p*-Dimethylstyrene	1452	t
1-Octen-3-ol	1452	0.4
trans-Sabinene hydrate	1474	0.3
α-Copaene	1497	t
β-Bourbonene	1535	0.1
cis-Sabinene hydrate acetate	1542	0.2
trans-*p*-Menth-2-en-1-ol	1571	0.2
β-Ylangene	1589	0.1
Bornyl acetate	1590	0.3
Terpinen-4-ol	1611	1.6
β-Caryophyllene	1612	0.7
cis-*p*-Menth-2-en-1-ol	1638	t
δ-Terpineol	1682	0.2
trans-Verbenol	1683	0.2
γ-Muurolene	1704	0.1
α-Terpineol	1706	0.7
Borneol	1719	2.2
Germacrene D	1726	0.2
β-Bisabolene	1741	0.4
cis-Piperitol	1758	t
δ-Cadinene	1773	0.1
γ-Cadinene	1776	t
Cumin aldehyde	1802	t
cis-Calamenene	1853	t
p-Cymen-8-ol	1864	0.2
Thymol acetate	1867	0.4
(*E*)-Nerolidol	2050	0.3
Humulene epoxide-II	2071	1.9
Cumin alcohol	2113	0.1
Isothymol (=2-Isopropyl-4-methyl phenol)	2181	0.2
Thymol	2198	52.2
Isocarvacrol (=4-Isopropyl-2-methyl phenol)	2221	0.2
Carvacrol	2239	3.6
	Total	**99.6**

[a] *Relative retention indices (RRI) calculated against n-alkanes.*
[b] *The contents (%) of the individual components were calculated based on the peak area (FID response).*
[c] *t: Trace (< 0.1 %).*

As shown in Table 2, the essential oil had significant scavenging effect on ABTS, H_2O_2 and superoxide radical at 60 µg/mL with percentage inhibition of 100, 100 and 77.20%, when compared with that of thymol 97.08, 93.27, 61.72% and trolox 97.89, 81.72, 61.36%, respectively. Fe^{2+} chelating ability of the essential oil and thymol at 60 µg/mL were determined as 17.33 and 21.42%. The total antioxidant activity of the essential oil and thymol were measured by the inhibition of lipid peroxidation with percentage inhibition of 41.44 and 42.24% which were comparable to that of the

standard compound (44.71%). According to the present study, the essential oil of *T. haussknechtii* was found to be an effective antioxidant according to different *in vitro* assays when it was compared to standard antioxidant compound trolox. The effect was considered to be due to its major component thymol which could play an important role in the antioxidant activity because of its phenolic structure [11].

Table 2. Antioxidant activity of the essential oil of *T. haussknechtii* at 60 µg/mL.

	Inhibition (%)[a]		
	Essential Oil	**Thymol**	**Trolox**
ABTS radical cation decolorization activity	100.00 ± 0	97.08 ± 1.01	97.89 ± 0.21
DPPH free radical scavenging activity	3.24 ± 1.81	23.66 ± 0.29	45.92 ± 0.32
Ferrous ion chelating activity	17.33 ± 2.43	21.42 ± 0.68	59.83 ± 0.19
Superoxide anion radical scavenging activity	77.20 ± 1.17	61.72 ± 1.42	61.36 ± 1.03
Total antioxidant activity	41.44 ± 0.52	42.24 ± 1.15	44.71 ± 1.27
H_2O_2 scavenging activity	100.00 ± 0	93.27 ± 0.84	81.72 ± 0.41

[a] *% inhibition (means ± SD)*

The microplate assay for anticholinesterase activity: Inhibitory activities of AChE and BuChE of the essential oil were evaluated by colorimetric Ellman's method [22] with some modifications using commercially available neostigmine bromide as the reference compound [23]. In this study, the essential oil of *T. haussknechtii* showed anticholinesterase activity against acetylcholinesterase with 57.33% and butyrylcholinesterase with 40.11% inhibition at 25 µg/mL concentration (Table 3). The essential oil obtained from *T. haussknechtii* was rich in thymol (52.2%) and thymol exhibited strong acetyl- (83.0%) and butyrylcholinesterase (98.0%) inhibitory activities. Therefore, the anticholinesterase activity of the essential oil could be attributed to its major constituent thymol [24]. We present the first report on anticholinesterase activity of the essential oil of *T. haussknechtii* in this study.

Table 3. *In vitro* AChE and BuChE inhibition of the essential oil of *T. haussknechtii* at 25 µg/mL.

	AChE[a] (%)	**BuChE[b] (%)**
Essential oil	57.33 ± 3.05	40.11 ± 2.08
Thymol	83.0 ± 0.11	98.0 ± 1.38
Neostigmine bromide	100 ± 0	100 ± 0

[a] *50% inhibitory concentration (means ± SD of three experiments) of AChE.*
[b] *50% inhibitory concentration (means ± SD of three experiments) of BuChE.*

It can be concluded that the essential oil of *T. haussknechtii* possess strong antioxidant effect and anticholinesterase activity which may be due to its high phenolic content. These results indicate that *T. haussknechtii* could be a good source for natural antioxidants which were very important in prevention of many disease and protection of health.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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