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Chemical Composition, Antioxidant and Anticholinesterase Activities of the Essential oil of *Origanum rotundifolium* Boiss. from Turkey

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Abstract: The essential oil was obtained by hydrodistillation from the aerial parts of *Origanum rotundifolium* Boiss. Its chemical content and composition were analyzed by using a gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Total phenolic content of the essential oil was determined as 132.39 µg gallic acid equivalent by Folin–Ciocalteu's method and the major component was identified as carvacrol (56.8 %) along with *p*-cymene (13.1 %), (*Z*)-β-ocimene (5.4 %), β-caryophyllene (3.9 %), borneol (3.4 %) and thymol (3.2 %). After chemical characterization, the essential oil was evaluated for its antioxidant activity by DPPH free radical, superoxide anion radical and hydrogen peroxide scavenging activities as well as ferrous ion-chelating power test, ABTS radical cation decolorization assay and ferric thiocyanate methods. Besides antioxidant activity, acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil were also evaluated by Ellman's method. It demonstrated inhibitory activities on AChE and BuChE, key enzymes in the pathogenesis of Alzheimer's disease (AD), in addition to significant antioxidant activity.

Keywords: *Origanum rotundifolium*; Essential oil; Antioxidant; Acetylcholinesterase; Butyrylcholinesterase.

1. Plant Source

The genus *Origanum* is a member of the Lamiaceae family and is represented in the flora of Turkey by 24 species and 6 hybrid species [1-3], some of which have been used as sedative, diuretic, diaphoretic, antidiarrheal, digestive and carminative in Turkish folk medicine [4]. *O. rotundifolium* is a narrowly distributed species that is mainly confined in northeast of Turkey to west of Transcaucasus. [5]. The essential oil of *O. rotundifolium* has been reported to show a variety of biological activities such as antibacterial [6], antigenotoxic, antioxidant [7], antiprobiotic [8] and antimicrobial [9]. In this study, first the chemical composition and total phenolic content of the essential oil from aerial parts of *O. rotundifolium* were determined, and then, its antioxidant capacity, acetylcholinesterase and butyrylcholinesterase inhibitory activities were evaluated.

The aerial parts of wild *Origanum rotundifolium* Boiss. were collected from Erzurum Taşağıl Village (2400 m) in August 2012 and identified by Mehmet Önal from Regional Directorate of

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Forestry, Turkey. The voucher specimen (AUEF 1002) has been deposited in the Herbarium of the Faculty of Pharmacy, Atatürk University, Erzurum, Turkey.

2. Previous Studies

Previously, various chemical compositions of *O. rotundifolium* essential oils were studied. The main constituents of the oil obtained from the aerial parts of this species collected from Ardanuç (Turkey) were cis-sabinene hydrate (21.53 %) and linalyl acetate (7.48 %) [10]. Major components of the oil obtained from the aerial parts of *O. rotundifolium* collected from Aşkale (Turkey) were thymol (40.86 %), carvacrol (43.62 %), *p*-cymene (5.95 %) and borneol (2.49 %) [7]. Oil of *O. rotundifolium* collected from İspir (Turkey) contained mainly carvacrol (54.6 %), p-cymene (12.5 %), borneol (5.9 %) and thymol (3.5 %) [9]. Major components of the oil obtained from the aerial parts of *O. rotundifolium* were collected from Borçka (Turkey) were borneol (28.21 %), *o*-cymene (9.55 %), terpinen-4-ol (8.64 %), and spathulenol (8.89 %) [11]. Variation in essential oil compositions can occur as a result of different soil conditions, altitude, climatic conditions, seasonal factors and other environmental features [12].

A few studies have been reported previously on the anticholinesterase activity of some *Origanum* species [13-16], but not for *O. rotundifolium*.

Table 1. Chemical composition of the essential oil from *O. rotundifolium*

Compound	RRI[a]	RRI[b]	%[c]
tricyclene	1014	1018 [17]	t[d]
α-pinene	1032	1032 [18]	0.7
α-thujene	1035	1035 [19]	0.5
camphene	1076	1077 [17]	0.8
β-pinene	1118	1117 [17]	0.1
sabinene	1132	1030 [17]	t
thuja-2,4(10)-diene	1136	1135 [20]	t
δ-3-carene	1159	1159 [18]	0.1
myrcene	1174	1174 [18]	1.1
α-terpinene	1188	1188 [18]	1.0
dehydro-1,8-cineole	1195	1195 [21]	t
limonene	1203	1203 [21]	0.2
o-mentha-1(7),5,8-triene	1224	1224 [22]	0.2
(*Z*)-β-ocimene	1246	1241 [17]	5.4
(*E*)-β-ocimene	1266	1258 [17]	0.1
3-octanone	1266	1266 [23]	1.2
p-cymene	1280	1278 [17]	13.1
terpinolene	1290	1288 [17]	0.2
3-octanol	1393	1400 [24]	0.1
trans-linalool oxide (furanoid)	1450	1450 [25]	t
α,*p*-dimethylstyrene	1452	1457 [26]	t
1-octen-3-ol	1452	1451 [27]	0.6
trans-sabinene hydrate	1474	1474 [28]	0.1
cis-linalool oxide (Furanoid)	1478	1478 [25]	t
α-copaene	1497	1495 [17]	0.1
β-bourbonene	1535	1535 [28]	0.1
linalool	1553	1553 [28]	0.4
cis-sabinene hydrate	1556	1555 [28]	t
linalyl acetate	1565	1565 [29]	0.1
trans-*p*-menth-2-en-1-ol	1571	1571 [28]	t
β-ylangene	1589	1589 [29]	0.1
terpinen-4-ol	1611	1611 [28]	1.1
β-caryophyllene	1612	1612 [28]	3.9
carvacrol methyl ether (=methyl carvacrol)	1614	1604 [30]	0.2
trans-dihydrocarvone	1624	1616 [31]	0.1
aromadendrene	1628	1611 [17]	0.5
cis-isodihydrocarvone	1645	1645 [22]	0.2
alloaromadendrene	1661	1659 [26]	0.1
α-humulene	1687	1675 [17]	0.2
γ-muurolene	1704	1692 [17]	0.2
α-terpineol	1706	1694 [17]	0.3
borneol	1719	1717 [26]	3.4
α-muurolene	1740	1742 [26]	0.1
δ-cadinene	1773	1774 [26]	0.3
γ-cadinene	1776	1779 [26]	0.1
cadina-1,4-diene (=cubenene)	1799	1810 [26]	t
myrtenol	1804	1807 [26]	t
cis-calamenene	1853	1847 [26]	t
geraniol	1857	1859 [26]	t
p-cymen-8-ol	1864	1865 [26]	0.3
4-isopropyl salicylaldehyde	1940	1940 [32]	0.1
caryophyllene oxide	2008	2008 [21]	0.8
cumin alcohol	2113	2100 [21]	0.1
spathulenol	2144	2150 [33]	0.4
eugenol	2186	2186 [18]	0.2
thymol	2198	2198 [18]	3.2
isocarvacrol (=4-isopropyl-2-methyl phenol)	2221	2221 [34]	t
carvacrol	2239	2239 [34]	56.8
α-cadinol	2255	2255 [21]	t
caryophylla-2(12),6(13)-dien-5α-ol (=caryophylladienol II)	2324	2324 [35]	t
caryophylla-2(12),6-dien-5β-ol (=caryophyllenol II)	2392	2392 [35]	t
	Total		**98.9**

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

3. Present Study

Chemical composition of the essential oil: In this study, essential oil was obtained from the aerial parts of *O. rotundifolium* with 1.01 % (w/w) yield. The chemical composition of the essential oil was analyzed by GC-FID and GC-MS analyses. *O. rotundifolium* essential oil was characterized by the presence of sixty two compounds, representing 98.9 % of the total oil. Carvacrol was recognized as the main constituent (56.8 %) of the total oil composition along with *p*-cymene (13.1 %), (*Z*)-β-ocimene (5.4 %), β-caryophyllene (3.9 %), borneol (3.4 %) and thymol (3.2 %). The volatile components identified in the essential oil are presented in Table 1 along with their relative retention indices and 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, and then was programmed to 220°C at a rate of 4°C/min and kept in this temperature for 10 min. The oven temperature was 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 spectra 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 performed by using the same column and appropriate operational conditions.

Total phenolic content of the essential oil: The total phenolic content of the essential oil was determined by using the Folin-Ciocalteu method [36] as gallic acid equivalents using the equation that was obtained from the standard graph (S1). 132.39 ± 0.12 μg gallic acid equivalent of phenolic compounds were detected in 1 mg of essential oil of *O. rotundifolium*. The experiment was repeated at least three times.

Antioxidant activity: Antioxidant activity was evaluated by using six different tests: DPPH free radical scavenging activity [37], ferrous ion-chelating power test [38], ABTS radical cation decolorization assay [39], superoxide anion radical scavenging activity [40], total antioxidant activity by ferric thiocyanate method [41], hydrogen peroxide scavenging activity [42]. All experiments were repeated at least three times. The essential oil of *O. rotundifolium* exhibited significant antioxidant activity measured by scavenging of $ABTS^{\bullet+}$, H_2O_2, and superoxide radical and inhibition of linoleic acid oxidation. As shown in Table 2, the essential oil had scavenging effect on ABTS, H_2O_2 and superoxide radical at 60 μg/mL with percentage inhibition of 100, 100 and 68.65 %, when compared with that of trolox 97.89, 81.72 and 61.36 %, respectively. The total antioxidant activity of the essential oil was measured by the inhibition of lipid peroxidation with percentage inhibition of 44.06 %, which was comparable to that of the standard compound (44.71 %). A strong relationship between total phenolic content and antioxidant activity has been reported [11]. Therefore the activity observed for this essential oil should be related to its phenolic content.

Table 2. Antioxidant activity (%) of the essential oil of *O. rotundifolium* at 60 μg/mL

	Essential Oil[a]	**Trolox**[a]
ABTS radical cation decolorization activity	100.00 ± 0	97.89± 0.21
DPPH free radical scavenging activity	6.53 ± 0.98	45.92 ± 0.32
Ferrous ion chelating activity	21.67 ± 1.31	59.83 ± 0.19
Superoxide anion radical scavenging activity	68.65 ± 1.01	61.36 ± 1.03
Total antioxidant activity	44.06 ± 0.72	44.71 ± 1.27
H_2O_2 scavenging activity	100.00 ± 0	81.72 ± 0.41

[a] *% inhibition (mean ± SD)*

Anticholinesterase activity: Inhibitory activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) of the essential oil were evaluated by colorimetric Ellman's method [43], with some modifications using commercially available neostigmine bromide as the reference compound [44]. The experiments were repeated three times for consistency. The essential oil of *O. rotundifolium* showed anticholinesterase activity against acetylcholinesterase with 38.66 % inhibition, and butyrylcholinesterase with 50.66 % inhibition at 25 μg/mL concentration.

On the other hand, the major component carvacrol showed 15.36 and 23.16 % inhibition at the same concentration, respectively (Table 3). The essential oil was more effective than carvacrol. The

anticholinestrase activity of essential oils is strongly dependent on the interaction of different terpenoid contents, including synergic effect of monoterpenoids [45].

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

	AChE (%)[a]	BuChE (%)[a]
Essential oil	38.66 ± 1.33	50.66 ± 2.51
Carvacrol	15.36 ± 0.48	23.16 ± 0.23
Neostigmine bromide	100 ± 0	100 ± 0

[a] *% inhibition (mean ± SD)*

One of the most important causes of Alzheimer's disease (AD), an oxidative stress-induced neurodegenerative disease, is cholinergic loss in the brain. Specifically, a reduction in the amount of acetylcholine released from cholinergic synapses in Alzheimer's patients has been identified. A treatment method has been developed to increase or protect the amount of acetylcholine by inhibiting acetylcholinesterase [46]. Our study demonstrated that essential oil of *O. rotundifolium* has inhibitory activity on AChE and BuChE in addition to significant antioxidant activity. The use of antioxidants may be useful in the treatment of AD [47]. To the best of our knowledge, this is the first report on cholinesterase inhibitory activity of the essential oil of this plant.

Supporting Information

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

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