

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. © 2017 ACG Publications. All rights reserved.

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], antiprotective [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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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 μ m 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 μ g 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⁺, H₂O₂, and superoxide radical and inhibition of linoleic acid oxidation. As shown in Table 2, the essential oil had scavenging effect on ABTS, H₂O₂ and superoxide radical at 60 μ g/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 μ g/mL

	Essential Oil ^a	Trolox ^a
ABTS radical cation decolorization activity	100.00 \pm 0	97.89 \pm 0.21
DPPH free radical scavenging activity	6.53 \pm 0.98	45.92 \pm 0.32
Ferrous ion chelating activity	21.67 \pm 1.31	59.83 \pm 0.19
Superoxide anion radical scavenging activity	68.65 \pm 1.01	61.36 \pm 1.03
Total antioxidant activity	44.06 \pm 0.72	44.71 \pm 1.27
H ₂ O ₂ scavenging activity	100.00 \pm 0	81.72 \pm 0.41

^a % inhibition (mean \pm 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 μ g/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

anticholinesterase 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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