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Short communication

A Preliminary Study on the Antioxidant Activity of *Origanum haussknechtii* Boiss.

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The aim of this study was to determine antioxidant activity of the water and methanol extracts from the aerial parts of *Origanum haussknechtii* Boiss. (Lamiaceae), which is an endemic to Turkey. The water and methanol extracts of the plant were evaluated for their antioxidant capacity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, trolox equivalent antioxidant capacity (TEAC) and inhibition of lipid peroxidation with thiobarbituric acid (TBA) assays. In addition, the total phenolic content in both extracts was determined with spectrophotometric method. The extracts were found to have different levels of antioxidant properties in the test models used. Both extracts of the plant were shown to possess DPPH radical scavenging activity compared to reference substances, but that of methanol extract (IC_{50}=1.22 ± 1.25 mg/mL) was found to be more pronounced. In the TEAC assay, the methanol extract displayed higher antioxidant capacity (1.35 ± 0.28 mmol trolox/g) than the water extract. In the TBA method, the methanol extract (IC_{50}=23.62 ± 2.42 µg/mL) also showed higher inhibitory activity on the lipid peroxidation than the water extract when compared to propyl gallate. The results revealed that the methanol extract of *O. haussknechtii* is a good antioxidant source of natural origin.

Key words: Lamiaceae, *Origanum haussknechtii* Boiss., Plant extracts, Antioxidant activity, Total phenolics

Origanum haussknechtii Boiss.'nin Antioksidan Aktivitesi Üzerine Bir Ön Çalışma

Bu çalışmanın amacı Türkiye için endemik bir tür olan *Origanum haussknechtii* Boiss. (Lamiaceae)'nin toprak üstü kısımlarından hazırlanan sulu ve metanollü ekstrelerin antioksidan aktivitesinin tayin edilmesidir. Ekstreler antioksidan kapasiteleri için 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürücü, troloks'a eşdeğer antioksidan kapasite (TEAC) ve tiyobarbitürik asit (TBA) testi ile lipid peroksidasyonun inhibisyonu bakımından değerlendirilmiştir. Ayrıca ekstrelerin total fenolik içerikleri spektrofotometrik metot ile tayin edilmiştir. Ekstrelerin kullanılan test modellerinde farklı düzeylerde antioksidan özelliklere sahip olduğu bulunmuştur. Her iki ekstrenin referanslar ile kıyaslandığında DPPH radikal süpürücü aktiviteye sahip olduğu görülmüştür, bununla birlikte metanollü ekstrenin (IC_{50}=1.22 ± 1.25 mg/mL) daha belirgin aktiviteye sahip olduğu bulunmuştur. TEAC testinde, metanollü ekstrenin (1.35 ± 0.28 mmol trolox/g) sulu ekstreden daha yüksek antioksidan kapasiteye sahip olduğu bulunmuştur. TBA metotunda da metanollü ekstre (IC_{50}=23.62 ± 2.42 µg/mL) propil gallat ile karşılaştırıldığında lipid peroksidasyon üzerine sulu ekstreden daha yüksek inhibitör aktivite göstermiştir. Sonuçlar *O. haussknechtii*'den hazırlanan metanollü ekstrenin doğal orijinli iyi bir antioksidan kaynağı olduğnu göstermiştir.

Anahtar kelimeler: Lamiaceae, *Origanum haussknechtii* Boiss., Bitki esktreleri, Antioksidan aktivite, Total fenolikler

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INTRODUCTION

Free radicals play a vital role in the pathogenesis of various human diseases and therefore, antioxidants have an important function in the prevention of these diseases (1). Many medicinal plants, vegetables and spices are promising and diverse sources of natural antioxidants, which have been found to be excellent sources of antioxidant compounds such as phenolic or nitrogen-containing compounds and carotenoids (2-5). Therefore, as well-known, a great number of medicinal plants have been investigated for their antioxidant potentials for human health. The family Lamiaceae has been particularly investigated in regarding natural antioxidants, e.g. mint, rosemary, sage, oregano and thyme, have been shown strong antioxidant activity (5-9).

Origanum haussknechtii Boiss. (Lamiaceae) is an endemic species to Turkey, which can grow up to 50 cm long (10). It has been used as a tea in Erzincan-Kemaliye region of the country (Personal data). To date, there has been only one report on the composition of the essential oil of *O. haussknechtii* (11). To the best of our knowledge, no biological data on the plant was found according to a literature survey. The aim of this study was to determine preliminary antioxidant properties of the plant. Therefore, we evaluated the antioxidant capacity of the water and that the water and methanol extracts of the plant in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, trolox equivalent antioxidant capacity (TEAC) and thiobarbituric acid (TBA) assay.

MATERIAL AND METHODS

Plant material

Origanum haussknechtii Boiss. (Lamiaceae) was collected from the vicinity of Kemaliye, Erzincan, Turkey in August 2008. The voucher specimen was authenticated by Prof. Dr. Mecit Vural, Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey. Authenticated voucher specimen was deposited in the Herbarium of the Gazi University (GAZI), Ankara, Turkey.

Preparation of plant extracts

The aerial parts of plant material were air dried until dryness at room temperature and under shade, and then powdered to a fine grade by using a laboratory scale mill.

Preparation of MeOH extract: 20 g of the powdered plant material were extracted two times with 200 mL of MeOH at room temperature with magnetic stirrer for 8 hours. Following filtration, the combined methanolic extract was evaporated to dryness *in vacuo* to give a crude extract (3.12 g, 5.76% w/w). The crude extract was dispersed in water and lyophilized.

Preparation of water extract: 5 g of the powdered plant material were boiled with 100 ml distilled water for 30 min. The water extracts were filtered when hot and then the resultant extracts were lyophilized (1.15 g, 15.62% w/w).

Determination of total phenols

Total phenol content of *O. haussknechtii* methanol and water extracts was determined using the Folin-Ciocalteu technique (12). To 50 µL sample were added 250 µL of undiluted Folin-Ciocalteu reagent. After 1 min, 750 µL of 20 % (w/v) aqueous Na_2CO_3 were added, and the volume was made up to 5.0 mL with H_2O. After 2 h incubation at 25ºC, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The total phenolic contents were determined as gallic acid equivalents (mg gallic acid/g extract).

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Brand-Williams et al. (13). A 0.75 mL of methanol solution of the extracts at various concentrations was mixed with 1.5 mL of a DPPH methanolic solution (20 mg/L). After 20 min incubation in darkness and at ambient temperature, the absorbance was recorded at 517 nm. The percent scavenging of DPPH was calculated using the formula:

% scavenging activity = [1 - (ABS_{sample} / $ABS_{control}$)] x 100,

where $ABS_{control}$ is the absorbance of the control reaction (containing all reagents except the test samples), and ABS_{sample} is the absorbance of the sample extracts / references.

BHT and quercetin were used as positive controls. The decolorization was plotted against the sample extract concentration, and a linear regression curve was established in order to calculate the IC_{50} (mg/mL) which is the amount of sample necessary to decrease by 50% the absorbance of DPPH.

Trolox equivalent antioxidant capacity (TEAC)

The scavenging activity of the samples on 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) radical cation was carried out according to the method of Re et al. (14). Briefly, $ABTS^{\cdot+}$ radical cation was generated by a reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature and used within 2 days. The $ABTS^{\cdot+}$ solution was diluted with methanol to an absorbance of 0.700±0.020 at 734 nm. All samples were diluted appropriately to provide 20-80% inhibition of the blank absorbance. 10 µL of the diluted sample were mixed with 1 mL of diluted $ABTS^{\cdot+}$ solution. The assay with the mixture was carried out in triplicate, the mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. Trolox solution (final concentration 0.50-2.25 mmol/L) was used as a reference standard. The results were expressed as mmol trolox/g dry weight of plant material.

Thiobarbituric acid test (TBA) test for antioxidant activity using liposomes

The efficacy of inhibiting lipid peroxidation of the extracts was determined according to the method described by Güvenç et al. (15). Different concentrations of the extracts (0.016-1 mg/mL) and propyl gallate (0.000064-1 mg/mL) as reference were tested for their lipid peroxidation activity against liposomes prepared from bovine brain. The absorbance was measured at 532 nm. The inhibition of lipid peroxidation was measured as follows:

% inhibition = 100 x (FRM - B) - (ET- B - EA) / (FRM - B)

where FRM is the absorbance of the full reaction mixture (liposomes and iron source plus solvent water without the test substance), B is the absorbance of the blank mixture (liposomes only), ET is absorbance of the extract test mixture (full reaction mixture plus test substance), EA is the absorbance due to the extract alone. The IC_{50} value of the extracts was calculated by linear regression analysis.

RESULTS and DISCUSSION

The antioxidant activity of *O. haussknechtii* was evaluated for the first time in this study. Two different solvent extracts, methanol and water, were prepared from the aerial parts of the plant and their antioxidant effects for their antioxidant capacity in DPPH radical scavenging, trolox equivalent antioxidant capacity and inhibition on lipid peroxidation using by thiobarbituric acid assay. The extracts yields (w/w) are given as follows: water extract (5.76%) and methanol extract (15.62%).

The methanol and water extracts were found to have different levels of antioxidant properties in the test models used (Table 1). Both extracts of the plant were displayed to possess scavenging activity on DPPH radical compared to reference substances such as BHT and quercetin, but that of methanol extract (IC_{50}=1.22 ± 1.25) was found to be more prominent. In the TEAC assay, the methanol extract exerted the higher antioxidant capacity (1.35 ± 0.28 mmol trolox/g) than the water extract (1.22 ± 0.55 mmol trolox/g) of the plant. The methanol extract also showed higher antioxidant activity (IC_{50}=23.62 ± 2.42 µg/mL) in TBA test using the lipid peroxidation of liposomes than the water extract (IC_{50}=98.99 ± 1.58 µg/mL) when compared to propyl gallate (IC_{50}=0.24 ± 0.01 µg/mL). In addition, the amount of total phenolics in both extracts was determinated by the Folin-Ciocalteu method. The total phenolic contents were expressed as gallic acid equivalents (mg gallic acid/g extract). The amounts of total phenolics were found to be 309.25±1.99 mg/g in the water extract and 191±1.37 mg/g in the methanol extract.

Table 1. Antioxidant activities and total phenolic contents of the extracts of *O. haussknechtii.*

Samples	Total phenolic content[a]	DPPH radical scavenging effect[b]	TBA[c]	TEAC[d]
Water extract	309.25±1.19	1.66 ± 0.42	98.99 ± 1.58	1.22 ± 0.55
Methanol extract	191.00±1.37	1.22 ± 1.25	23.62 ± 2.42	1.35 ± 0.28
Quercetin		0.06 ± 0.35		
BHT	-	0.50 ± 0.17	-	-
Propyl gallate	-	-	0.24 ± 0.01	-

Results were presented as mean ± standard error mean (S.E.M.) (n=3)
BHT, quercetin, propyl gallate and ascorbic acid were used as positive controls.
[a] Total phenolic content was expressed as mg gallic acid/g extract
[b] Values expressed as IC_{50} (mg/mL)
[c] Values expressed as IC_{50} (μg/mL)
[d] Values expressed as mmol trolox/g extract.

The antioxidant properties of different extracts (CH_3OH, Et_2O, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O, etc.) obtained from several *Origanum* species (*O. vulgare*, *O. heracleoticum* and *O. majorana*) have been investigated in previous studies. In these studies, *Origanum* species were found to have different levels of antioxidant effects in tested *in vitro* antioxidant methods. The observed differences in antioxidant activity were reported to be attributed mainly by the levels of phenolic acids and flavonoids in the investigated *Origanum* extracts (9, 16-18). *Origanum* species have been reported to possess a rich phenolic content, especially rosmarinic acid, which was found as the predominant compound among the other identified phenolic compounds (9, 19). In another study, HPLC analysis of the methanol extract of *O. majorana* revealed the presence of gallic, chlorogenic, caffeic, *p*-coumaric, ferulic, rosmarinic acids and methyl rosmarenate as phenolic acids, besides some flavonoids such as apigenin and luteolin-7-*O*-rutinoside. Among them, apigenin and methyl rosmarenate were found to be in the major quantities in the methanol extract of *O. majorana* (18).

Up to date, no bioactivity data has been reported on *O. haussknechtii*. To the best of our knowledge, the only phytochemical study carried out was on the essential oil composition of *O. haussknechtii* which was shown to contain *p*-cymene (15.56 %) and borneol (14.24 %) as major compounds (11). In our study, the methanol extract was applied to TLC. Spraying of TLC plates with a DPPH alcoholic solution showed that all spots corresponding to phenolic compounds reacted with the DPPH reagent. Phenolic compounds are called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals (20). The phenolic compounds in the methanol extract of *O. haussknechtii* could be responsible for antioxidant activity in the present study

Our results showed for the first time that methanol extract of *O. haussknechtii* aerial parts possesses a good antioxidant activity. The plant may be considered as a good source of natural antioxidants for medicinal uses such as against aging and other diseases related to free radical mechanisms. Further investigations on *O. haussknechtii*, in order to reveal the compounds responsible from the antioxidant activity are in progress.

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