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Antioxidant Capacity of Methanol Extract of Turkish Endemic Species *Origanum haussknechtii*

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ABSTRACT: The aim of this study is to reveal antioxidant capacity of methanol extract of *Origanum haussknechtii* aerial parts. The antioxidant capacity of methanol extract were assayed with various methods, metal chelating capacity, DPPH free radical and ABTS radical cation scavenging activity, including total phenolic compound contents by Folin – Ciocalteu reagent (FCR). The obtained results were compared with standard antioxidants such as Ascorbic acid, BHT and EDTA. The methanol extract of *O.haussknechtii* aerial parts showed free radical scavenging activity similar to that of BHT. Also, this extract exhibited strong ABTS radical cation activity. As a conclusion of this study, the methanol extract of *O.haussknechtii* has beneficial effects on metal chelating, ABTS radical cation , DPPH radical scavenging abilities and may thus exert protection against oxidative damage.

KEYWORDS: *O.haussknechtii,* DPPH, ABTS, metal chelating, total phenolic contents.

1 INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. In general, there are two basic categories of antioxidants, natural and synthetic [1].

Synthetic antioxidants, such as butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT) and tert-butyl hydroquinone (TBHQ), are widely used in food industry to retard or minimize oxidative deterioration of foods. However, consumers have rejected synthetic antioxidants due to their suspected toxic and carcinogenic effects [2].

Natural antioxidants properties are mainly attributed to their phenolic contents, thus their antioxidants action is similar to synthetic phenolic antioxidants. Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents [3]. Therefore, natural antioxidants can protect the human body from free radicals and could retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [2].

The genus *Origanum* (Lamiaceae) is represented throughout the world by 41 species, and in Turkey by 23 species or 32 taxa, 21 being endemic to Turkey [4]. Members of the genus *Origanum* (Lamiaceae family) are among the most important aromatic plants in worldwide. Many *Origanum* species are characterized by a wide range of volatile secondary metabolites and by the existence of chemical differences with respect to both essential oil content and composition [5].

Origanum species are traditionally used as a spice and furthermore, they are used as an expectorant, antispasmodic, sweater, antiseptic in the treatment of gastrointestinal and respiratory tract diseases. *Origanum* species have recently been great of interest, in both academia and the food industry as potential natural additives, to replace synthetic products because of their good antimicrobial and antioxidant properties [4,6].

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Origanum haussknechtii Boiss. (Lamiaceae) is an endemic species to Turkey, which can grow up to 50 cm long [7]. In Turkish folk medicine, the leaves of *Origanum haussknechtii* are used as herbal tea [8].

The aim of this study is to reveal antioxidant activity of methanol extract from *Origanum haussknechtii* aerial parts. The antioxidant activity of methanol extract were assayed with various methods, metal chelating, ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, including total phenolic compound contents by Folin – Ciocalteu reagent (FCR).

2 MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Origanum haussknechtii Boiss. aerial parts were collected from Erzincan, Turkey and was identified by Assist. Prof. Dr. Narin Sadıkoğlu. Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, İnönü University, herbarium code numbers: *Origanum* 2009/002.

PREPARATION OF THE EXTRACTS

The aerial parts of *Origanum haussknechtii* were extracted with methanol by maceration at room temperature for 7 days stirring several times until the solvent become colorless. The liquid combined extracts were filtered and evaporated to dryness under reduced pressure at 50 ^{o}C in a rotary evaporator. The crude extract was then transferred to vial and kept at +4 ^{o}C. This crude extract was dissolved in solvent (methanol) and used for the assessment of antioxidant activity.

DPPH• RADICAL SCAVENGING ACTIVITY

The DPPH• radical scavenging activity of methanol extract from *O.haussknechtii* aerial parts was measured by the DPPH• method proposed by Wei et al. [9]. According to the results of scanning the spectrum obtained in this study, DPPH• exhibited a strong absorption band (λmax) at 517 nm. A solution of DPPH• in methanol (0.1 mM) was prepared and 3.9 mL of this solution was added to 0.1 mL of extract in solution across a variety of concentrations. After thirty minutes, the absorbance value was read at 517 nm. A lower absorbance shows a higher occurence of DPPH• radical scavenging activity. The IC_{50} value is inversely correlated to antioxidant ability of extract. A lower IC_{50} value reveals higher antioxidant activity.

$ABTS^{\bullet+}$ RADICAL CATION SCAVENGING ACTIVITY

The $ABTS^{\bullet+}$ assay was performed according to the method developed by Re et al. [10]. This assay is based on the formation of the free radical cation $ABTS^{\bullet+}$ by reaction of ABTS aqueous solution (7mM) with $K_2S_2O_8$ (2.45 mM), at room temperature, under darkness, for 12–16 hours. This stock solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. The reaction mixture comprised 3.96 mL of $ABTS^{\bullet+}$ solution and 0.04 mL of the extract at a variety of concentrations. After six minutes, the absorbance value was read off at 734 nm.

METAL CHELATING ACTIVITY

The ferrous ions (Fe^{2+}) chelating assay was performed according to the method developed by Dinis et al. and modified slightly [11]. The extract and standard (EDTA) (200µL) were added to 50 µL of $FeCl_2$ (2 mM) solution. The reaction was initiated by the addition 200 µL of ferrozine (5 mM), left standing at ambient temperature for five minutes. Then, methanol was added to this mixture until a final volume of 4 mL was achieved. According to the results of scanning the spectrum obtained in this study, the absorbance of the this mixture showed a strong absorption band (λmax) at 562 nm. The mixture was left standing at ambient temperature for a further ten minutes. Then, the absorbance was measured at 562 nm.

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

The amount of total phenolic compounds in the *O.haussknechtii* methanol extract was determined according to the method of Slinkard and Singleton [12]. 0.1 mL of extract solution was diluted with distilled water (4.6 mL). 0.1 mL of Folin-Ciocalteu reagent (diluted 1:3, v/v) was added. Then, 3 mL of Na_2CO_3 (2.0 %) were added and the mixture was left standing at ambient temperature for 2 hours. The absorbance value was read at 760 nm. Results were expressed as milligrams of total

phenolics per gram extract (mg GAE/g extract). The calibration equation for gallic acid was absorbance = 2.395x – 0.027 (R^2 = 0.9995).

STATISTICAL ANALYSIS

All data are the average of analyses in triplicate. The data were recorded as mean ± standard deviation and analyzed by the Graphpad Prism 5 Demo. The significance of the difference between means was determined by Tukey's Multiple Comparison Test ($P < 0.05$).

3 RESULTS AND DISCUSSION

TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY

The antioxidant activity of methanol extract from *O.haussknechtii* aerial parts was assayed with various methods, metal chelating, ABTS radical cation and DPPH free radical scavenging activity, including total phenolic compound contents by Folin – Ciocalteu reagent (FCR). The results obtained in this study are summarized in Table 1.

Table 1. Metal chelating, DPPH radical scavenging, ABTS radical cation scavenging activities and the amount of total phenolic compounds (PC) (as gallic acid equivalents) of methanol extracts from O.haussknechtii aerial parts.

Extracts/ Standarts	Metal chelating activity (%)	DPPH (IC_{50}: mg/mL)	ABTS (IC_{50}: mg/mL)	PC (mg/g extract)
Methanol extract	31.14 ± 0.8 [a]	0.32±0.02 [a]	0.75± 0.04 [a]	210.3 ± 0.5
EDTA	96.19± 0.7 [b]			
BHT		0.32± 0.03[b]		
Ascorbic acid		0.09±0.006[c]	0.19 ± 0.005[b]	

These values were the mean values of three replicates ± standard deviation. Different superscript letters in each column exhibit significant differences in mean values at P< 0.05 according to Tukey's Multiple Comparison test.

The free radical scavenging capacity of methanol extract of the plant was measured by DPPH assay. Butylated hydroxytoluene and ascorbic acid were used as standards. The DPPH radical scavenging capacity of the methanol extract and standards showed the following order: ascorbic acid (IC_{50}:0.09±0.006 mg/mL) > BHT (IC_{50}:0.32±0.03 mg/mL) = methanol extract (IC_{50}: 0.32±0.02 mg/mL). The methanol extract of *O.haussknechtii* aerial parts showed free radical scavenging activity similar to that of BHT.

The percentages of ferrous ion chelating capacity of mg/mL concentration methanol extracts from *O.haussknechtii* aerial parts and EDTA are shown in Table 1. The metal chelating activity of the methanol extract and the standard compounds at the mg/mL concentration are exhibited in the following order: EDTA (96.19±0.7%)> methanol extract (31.14±0.8%). The methanol extract showed lower metal chelating capacity than EDTA.

ABTS radical cation assay was used for the antioxidant capacity of plant extract. The ABTS radical cation scavenging capacity of methanol extract was measured according to the method of Re et al. [10]. Ascorbic acid was used as a standard. The ABTS radical cation scavenging activity of methanol extract is shown in Table 1. The ABTS radical cation scavenging effects of the methanol extract and standard are in the following order; ascorbic acid (IC_{50}: 0.19±0.005 mg/mL)>methanol extract (IC_{50}: 0.75±0.04 mg/mL). The methanol extract showed lower ABTS radical cation activity than ascorbic acid.

The total phenolic compounds in the methanol extract were determined from the regression equation of calibration curve (Y = 2.395x – 0.027 (R^2 = 0.9995) and expressed in gallic acid equivalents (GAE). The amount of total phenolic compounds in methanol extract are shown in Table 1.

On the other hand, to the best of our knowledge, there are only two reports in literature on the endemic *Origanum haussknechtii* [7,13]. It was reported that the composition of the water-distilled essential oil of *O.haussknechtii* was analysed by GC and GC/MS. p-cymene (15.56 %) and borneol (14.24 %) were characterised as the main constituents. Also, the antioxidant activities of water and methanol extracts from *O.haussknechtii* have been reported before [7]. In this study, the methanol extract of *O. haussknechtii* showed higher TBA test using the lipid peroxidation of liposomes and trolox equivalent antioxidant (TEAC) activity than the water extract. However, in our current study, the antioxidant capacity of methanol

extract of *O.haussknechtii* were assayed with different methods; DPPH free radical scavenging activity, metal chelating activity and ABTS radical cation scavenging activity. The antioxidant activity of methanol extract according to the results obtained from this study showed similar to with the literature [7]. Bioactive compounds in methanol extract were not identified in this study. In further studies, the phenolic compounds in methanol extract will be identified by ESI-Q-TOF LC/MS. The methanol extract of *O. haussknechtii* could be useful as an antioxidant agent in the future.

4 CONCLUSION

The methanol extract of *O.haussknechtii* aerial parts showed free radical scavenging activity similar to that of BHT. Also, this extract exhibited strong ABTS radical cation activity. Therefore, after examining the toxic effects on different normal cell line of the methanol extract, it is believed that this extract might be a potential source of antioxidant agents.

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