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Essential oil composition and antioxidant activity of endemic *Marrubium parviflorum* subsp. *oligodon*

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ABSTRACT

In this study, chemical composition and antioxidant activity of the essential oil and solvent extracts (hexane, dichloromethane, ethyl acetate, methanol and water) obtained from aerial parts of the endemic plant *Marrubium parviflorum* subsp. *oligodon* collected from Afyonkarahisar province in Turkey were examined. The antioxidant ability of solvent extracts and essential oil was evaluated using various methods (β-carotene-linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and reducing power assays) and the content of total phenolics and flavonoids were determined. In the essential oil, thirty-one compounds were identified by GC/MS analysis. The major components were (*Z,Z*)-farnesyl acetone (19.28%), caryophyllene oxide (15.85%) and pulegone (7.15%). In all assays, water extract showed the strongest antioxidant properties due to the highest content of total phenolics. The significantly high correlations were observed between the content of total phenolics and DPPH radical (0.894) and reducing power (0.983) assays. Methanol extract was the richest in total flavonoids. The present results support the traditional and possible use of the essential oil and extracts from *M. parviflorum* subsp. *oligodon* in food, pharmaceutical, and cosmetic industries.

1. Introduction

Biodiversity of flora, especially of endemic plant species, in Turkey is remarkable. Due to the richness of plant species, Turkey is one of the most important countries in the World in respect to endemic plants. These plants are distributed in three main phytogeographic regions namely Irano-Turanian, Mediterranean and Euro-Siberian (Bulut and Yilmaz, 2010).

The genus *Marrubium* is distributed in Irano-Turanian and the Mediterranean phyto-geographical regions (Aytac et al., 2012). This genus contains herbaceous annual and perennial plants. The genus comprises about 40 taxa all over the world (Akgul et al., 2008). In Turkey, the genus *Marrubium* is represented by 21 taxa, of which 12 are endemic (Aytac et al., 2012).

The importance of *Marrubium* L. is associated to its application in traditional and modern medicine. Some of the members of this family show antioxidant, vasorelaxant and hypotensive activity, while others are used as anticancer, anti-microbial, analgesic, anti-inflammatory or antidiabetic agents (Khanavi et al., 2005; Kharazian and Hashemi, 2017). Labdane diterpenes, phenylethanoid glycosides, flavonoids and sesquiterpenes are well-known constituents of *Marrubium* species (Argyropoulou and Skaltsa, 2012). Flavonoids and phenolic comprise a large number of compounds isolated from a variety of vascular plants. They play an important physiological role in plants, acting as antioxidants, antimicrobials, repellents, visual attractors, etc. (Pietta, 2000). The beneficial effects acquired from both phenolic and flavonoids have been associated with their antioxidant activity (Balasundram et al., 2006; Pietta, 2000).

Marrubium parviflorum subsp. *oligodon*, endemic to Turkey, is a perennial herbaceous plant and grows mainly over the Continental and Central Anatolia. It is also known by the local name “Dağ cayi” and is used in folk medicine in East Anatolian part of Turkey for treatment of colds and as an antipyretic (Altundag and Ozturk, 2011). Data about phytochemical composition of *M. parviflorum* subsp. *oligodon* are very limited, and extensive research of literature resulted only in the study of Bal et al. (1999).

This work aimed to examine the chemical composition of essential oil and solvent extracts obtained from aerial parts of the endemic plant *M. parviflorum* subps. *oligodon*. In addition, antioxidant properties of essential oil and solvent extracts by using various methods (β-carotene-linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and reducing power assays) were evaluated. Considering the lack of

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knowledge about chemistry of *M. parviflorum* subsp. *oligodon* collected from Aegean region the results obtained in this study could be assumed as the first report.

2. Materials and methods

2.1. Plant material

Aerial parts of *Marrubium parviflorum* Fisch. & Mey. subsp. *oligodon* (Boiss.) Seybold were collected from Gocen village, Cay-Afyonkarahisar-TURKEY on 23 June 2015 (1085 m, 38° 23′ 44″N 30° 43′ 40″E), authenticated by Dr. Olcay CEYLAN, and deposited at the Department of Biology, Mugla Sıtkı Koçman University (Mugla-TURKEY) under the accession no. O.5203.

2.2. Preparation of the extracts

The air-dried plant material (20 g) was consecutively extracted with hexane, dichloromethane, ethyl acetate, methanol and water (Ozer et al., 2010). Yields were found to be 1.25%, 1.20%, 0.75%, 5.62%, and 6.25% (w/w) for hexane, dichloromethane, ethyl acetate, methanol and water extracts, respectively.

2.3. Isolation and analysis of the essential oil

The air-dried and ground plant material (300 g) was submitted for 5 h to hydro-distillation by using a British-type Clevenger apparatus (Ozer et al., 2010). The essential oil yields were determined as 0.012% (v/w).

The constituents of the oil were analyzed by means of gas chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC–MS) by using the analytical conditions reported by Sarikurkcu et al. (2013).

The GC-FID analysis of the essential oil was performed using a Thermofinnigan Trace GC/A1300 (E.I.) equipped with a SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). Helium was the carrier gas, at a flow rate of 1 ml/min. Injector temperature was set at 220 °C. The program used was 50–150 °C at a rate of 3 °C/min, held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μl were injected manually and in the splitless mode. Quantitative data of the oil was obtained from FID area percentage data.

The GC–MS analysis of the essential oil was performed with a Thermofinnigan Trace GC/Trace DSQ/A1300 (E.I. Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at 3 °C/min, then held isothermal for 10 min and raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μl were injected manually in the splitless mode.

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7 N and TRLIB libraries spectra and published data (Adams, 2007).

2.4. Assay for total phenolics and flavonoids

The total flavonoids and phenolics in the extracts were determined by using the method described previously (Zengin et al., 2016; Zengin et al., 2015).

For total phenolic content, sample solution (0.25 ml) was mixed with diluted Folin-Ciocalteu reagent (1 ml, 1:9) and shaken vigorously. After 3 min, Na_2CO_3 solution (0.75 ml, 1%) was added and the sample absorbance was read at 760 nm after 2 h incubation at room temperature. Total phenolic content was expressed as equivalent of gallic acid.

For total flavonoid content, sample solution (1 ml) was mixed with the same volume of aluminum trichloride (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 ml) to methanol (1 ml) without $AlCl_3$. The sample and blank absorbance were read at 415 nm after 10 min incubation at room temperature. Absorbance of the blank was subtracted from that of the sample. Total flavonoid content was expressed as equivalent of quercetin.

2.5. Antioxidant capacity

Antioxidant activities of samples were investigated by using β-carotene-linoleic acid (Ozer et al., 2010), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging (Kose et al., 2010), and reducing power (Sarikurkcu et al., 2015; Tepe et al., 2011) assays.

For total antioxidant activity by β-carotene-linoleic acid method, a stock solution of β-carotene-linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade). Twenty-five microliter linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.5 ml of various concentrations (0.4–2.0 mg/ml) of the essential oil and the extracts in methanol and water were added. Emulsion system was incubated for 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixture was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared.

For 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, one milliliter of various concentrations (0.2–1.0 mg/ml) of the essential oil and the extracts in methanol and water were added to a 4 ml of DPPH radical solution in methanol (0.004%). The mixture was shaken vigorously and allowed standing for 30 min. Absorbance of the resulting solution was measured at 517 nm by a spectrophotometer.

For ferric reducing power, various concentrations (0.2–1.0 mg/ml) of the essential oil and the extracts in methanol and water (2.5 ml) dissolved in methanol and water (2.5 ml) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide. The mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added. The mixture was then centrifuged at 200xg for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank.

2.6. Statistical analysis

All the assays were carried out in triplicate. SPSS commercial version 22.0 was used for statistical analysis. Data were compared by one-way analysis of variance (ANOVA) with Tukey's ($\alpha = 0.05$). The Pearson linear correlation was used to evaluate the association between the results.

3. Results and discussion

3.1. Chemical composition of essential oil

Sesquiterpenes, with monoterpenes, are an important constituent of essential oils in plants. They are the most diverse group of isoprenoids. In this study, thirty-one compounds were identified and grouped as follows: oxygenated sesquiterpenes (37.14%), sesquiterpene hydrocarbons (23.98%), oxygenated monoterpenes (22.01%), and other (5.01%), which makes 88.14% of all compounds detected in the essential oil of the above-ground parts of *M. parviflorum* subps. *oligodon*