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RESEARCH ARTICLE

Antioxidant Capacity and Total Phenol Contents of *Bifora radians* Bieb.

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This paper is dedicated to the memory of Prof. Dr. Turhan BAYTOP

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Summary

In the present study, the total phenol contents and in vitro antioxidant activities of two extracts obtained from herb of Bifora radians were investigated. The phenol contents of the samples were determined using Folin-Ciocalteu's phenol reagent. The antioxidant activity of diethyl ether and methanol extracts were examined by two different techniques: qualitative and quantitative DPPH• (1,1-diphenyl-2-picryllhydrazyl radical) assays to detect the free radical scavenging effect and the TBA (thiobarbituric acid) assay to detect their liposome lipid peroxidation.

Key Words: *Bifora radians; Apiaceae; total phenol contents, antioxidant activity.*

Bifora radians Bieb.'ın Antioksidan Kapasitesi ve Total Fenol İçeriği

Özet

Bu çalışmada Bifora radians bitkisinden elde edilen iki ekstrenin total fenol içerikleri ve in vitro antioksidan aktiviteleri incelendi. Örneklerin fenolik içeriği Folin-Ciocalteu reaktifi kullanılarak tayin edildi. Dietileter ve metanol ekstrelerinin antioksidan aktiviteleri iki farklı yöntemle incelendi: kalitatif ve kantitatif DPPH• (1,1-difenil-2-pikrilhidrazil radikal) yöntemleri ile radikal süpürücü etki; TBA (tiyobarbitürik asit) testi ile de lipozom lipit peroksidasyonu tayin edildi.

Anahtar kelimeler: *Bifora radians, Apiaceae, Total Fenol, antioxidant aktivite.*

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INTRODUCTION

Plants have been used in many fields including nutrition, culinary, dyeing, cosmetics and other industrial purposes; furthermore, medicinal plants are of great importance in human life. Medicinal plants have been used for treatment of various diseases as traditional medicine by mankind for centuries (1, 2). Flora of Turkey is very rich and Apiaceae family is one of the most diverse families in Turkey. Species in Apiaceae family have economic value and they have been used to treat several diseases in traditional medicine (3).

The genus *Bifora* Hoffm. (Apiaceae=Umbelliferae) is represented by two species in Turkey, namely *Bifora testiculata* (L.) Sprengel and *B. radians* Bieb. (4). *B. radians* is an annual herb with typical aroma growing along the borders of the fields, especially in chalky soils of Central Anatolia and known locally as "yabani kişnişotu, küçük kişnişotu, kokarot or aşuti" (3-5). It is used for its stomachic and carminative properties in Turkey as traditional medicine. Furthermore, the aerial parts of the plant have been used to aromatize foods especially soups in Doğubeyazıt-Van (3). It was reported that *B. radians* was found to be rich in alkanals and alkenals (5, 6). Additionally, antimicrobial (7, 8) and insecticidal activity (8, 9) studies were carried out on this species.

The purpose of this study was to evaluate the total phenol content and antioxidant capacity of *B. radians*, which is a natural plant of Turkey.

MATERIALS AND METHODS

Plant material:

Bifora radians was collected in İncek (Ankara province) during the flowering periods. Voucher specimens are deposited at the Herbarium (AEF) of the Faculty of Pharmacy, Ankara University, Turkey, with herbarium numbers AEF 25932 and AEF 25944.

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Extraction and preparation of test solutions:

1- *Preparation of diethyl ether extract*: 20 g of the fresh herb of species was crushed and macerated in 200 ml of diethyl ether for four days at room temperature with magnetic stirrer and the extract was filtered. The extract was dried under vacuum by using rotary evaporator at 35 °C.

2- *Preparation of MeOH extracts*: The methanol extract was obtained after keeping the herb in diethyl ether. This plant sample was carried out with methanol (200 mL) at a temperature of 50 °C for 8 h (x 3), shaking continuously (700 rpm/min) in the dark. After filtrating, the methanol was evaporated to dryness by rotary evaporator (40 °C).

Determination of total polyphenols:

The total polyphenol content of *Bifora radians* extracts were determined by Folin-Ciocalteu method, referring to calibration curve of gallic acid, phenol compound used as a standard (10-12). 250 *µl* of Folin-Ciocalteu's phenol reagent was mixed with 50 *µl* of the samples, and 500 *µl* of 20 % water solution of Na_2CO_3 was added to the mixture. After incubating the samples at room temperature for 30 min, their absorbance values were measured at 765 nm (Shimadzu UV-1800). The total polyphenols were estimated as gallic acid equivalent (GAE) and expressed in mg GAE/g extract (dw) ± standard deviation (SD). The data were obtained from the average of three determinations.

Antioxidant activity

Free Radical Scavenging Activity. Free radical scavenging activity of *B. radians* extracts has been evaluated using the two different DPPH test:

Qualitative DPPH•: DPPH assay was used as a rapid thin layer chromatography (TLC) screening method to evaluate the antioxidant activity of the diethyl ether and methanol extracts of *B. radians* species due to free radical scavenging. DPPH• is a purple-colored stable free radical, which on reduction gives yellow-colored diphenyl picryl hydrazine. When it is sprayed onto a TLC plate, any antioxidant compound is seen as a yellow zone on a purple background. Using Wiretrol II micropipettes, 2 µL of 1 mg/ml diethyl ether and methanolic solutions were applied to the silica gel TLC plates (Merck, Darmstadt, Germany), which were sprayed with 0.2% DPPH solution in MeOH, left at 20 °C, and examined at 30 min after spraying (13).

Quantitative DPPH•: Free radical scavenging activity of *B. radians* extracts has been evaluated using the DPPH test (14). 0.1 ml of solution of tested extract (in seven different consentration 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml) was added to 2.9 ml of 10^{-4} M of daily prepared methanol DPPH solution and left to stand in water bath at 30°C. The absorbance at 517 nm was measured with a model UV-1800 spectrophotometer (Shimadzu) after 30 min. Propyl gallate was used as a reference compound in the both assays.

Results were expressed as radical scavenging activity percentage (%) of the DPPH, defined by the formula $[(A_o - A_s)/A_o]x100$, where A_o is the absorbance of the control and A_s is the absorbance in the presence of the sample or standard. The half-maximal inhibitory concentrations (IC_{50}) of the extracts were calculated by linear regression analysis. The assays were carried out in triplicate, and the results are expressed as mean values ± standard deviation (S.D.).

Thiobarbituric acid (TBA) test. The *in vitro* antioxidant activity tests were carried out by lipid peroxidation of liposomes, where TBA was used to assess the efficacy of the extracts to protect liposomes from lipid peroxidation. It can be measured and quantified spectrophotometrically, and the intensity of color is a measurement of MDA (malonyldialdehyde) concentration. In order to assess the efficacy of the extracts to protect liposomes from lipid peroxidation TBA test was used (13, 15, 16). Briefly, extracts were redissolved in diethyl ether and methanol and tested at different concentrations. Liposomes were prepared from bovine brain extract in phosphate buffered saline (PBS) (5 mg/ml). The extract test reaction mixture consisted of 0.2 ml of liposomes, 0.1 ml $FeCl_3$, 0.1 ml ascorbic acid (1mM), 0.5 ml PBS and 0.1 ml of the extract solution to be assessed. All test tubes were incubated at 37°C for 20 minutes. The TBA test was performed after 20 minutes of incubation by adding 0.1 ml of 2% butylated hydroxytoluene (BHT) in ethanol solution followed by 0.5 ml of 1% w/v thiobarbituric acid (TBA) in 50 mM NaOH and 0.5 ml of 25% HCI. The system was heated to 85°C for 30 minutes (13). The absorbance was determined spectrophotometrically at 532 nm (Shimadzu UV-1800). The percentage of lipid peroxidation inhibition was assessed by using the following formula: [(FRM-B)- (ET-B-EA)/(FRM-B)] x100, where FRM is the absorbance of the

control reaction and ET is absorbance in the presence of the sample. The absorbance of liposomes alone (B) and extract alone (EA) were also taken into account.

The half-maximal inhibitory concentrations (IC_{50}) of the extracts were calculated by linear regression analysis (13, 16). Concentrations of 1, 0.5, 0.25, 0.125, 0.0625, 0.031 and 0.016 mg/ml of diethyl ether and MeOH extracts were prepared to be used in TBA. Propyl gallate was used as a reference compound in seven different concentrations (1; 0.20; 0.04; 0.008; 0.0016; 0.00032; 0.000064 mg/ml).

RESULTS

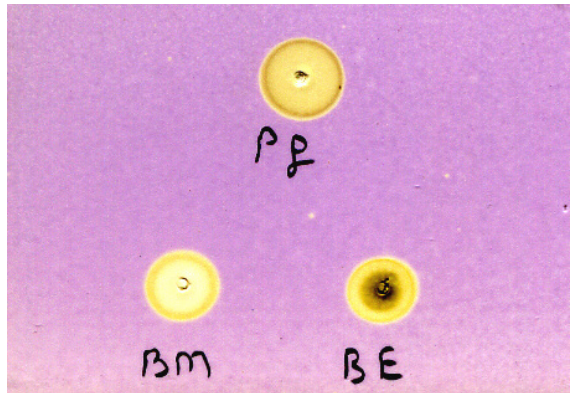
The results of total phenol contents obtained for *Bifora radians* extracts are given in Table 1.

Table 1. Total Phenol Contents of the Extracts of *Bifora radians*.

Species	Total phenol contents mg/g ± SD	
	Diethyl ether extracts	Methanol extracts
Bifora radians	96.67 ± 1.84	49.93 ± 1.04

The results of the qualitative DPPH test demonstrated that the extracts of the *Bifora* display prominent antioxidant activity (Fig. 1).

Figure1: Antioxidant activity by qualitative DPPH test on



TLC of *Bifora radians*. **Pg:** Propyl gallate; **BM:** Methanol extracts of *B. radians*; **BE:** diethyl ether extracts of *B. radians*.

The results of the qualitative DPPH test demonstrated (Table 2) that the extracts of the *Bifora radians* display significant antioxidant activity.

Table 2. Antioxidant activity by quantitative DPPH of the extracts of *Bifora radians*

Species	IC_{50} values (mg/ml) ± SD	
	diethyl ether extracts	Methanol extracts
Bifora radians	69.91 ± 1.03	43.21 ± 0.14
Propyl gallate	0.02 ± 0.04	

The antioxidant activities of the *B. radians* extracts on liposomes obtained from the TBA test are given in Table 3.

Table 3. Antioxidant Activities of *Bifora radians* in the TBA Test

Species	IC_{50} value (µg/ml) ± SD	
	diethyl ether extracts	Methanol extracts
Bifora radians	261.32 ± 2.72	89.43 ± 3.09
Propyl gallate	0.09 ± 0.18	

DISCUSSIONS

In the present study, we evaluated the total phenol contents of the diethyl ether and MeOH extracts of *Bifora radians* collected in Ankara province. We also demonstrated that the free radical scavenger activities and detected the liposome lipid peroxidation of these extracts using the TBA assay. Furthermore, total phenol content in the extracts were determined by the Folin-Ciocalteu's method as gallic acid equivalents (GAE). This investigation is the first report on the comparative analysis of total phenol and antioxidant activity of the different polarity extracts and of the *B. radians* naturally growing in Turkey.

According to the results obtained from the determination of total phenol contents, it was found that the diethyl ether extract include more phenol contents than the MeOH extract.

In the free radical scavenging activity test, yellow zones on a purple background were prominent for the both extracts of the *B. radians* and propyl gallate.

The antioxidants react with the DPPH, and convert it to the yellow-coloured diphenylpicrylhydrazine; the degree of discoloration indicates the amount of DPPH scavenged (11). The IC_{50} values of the MeOH and diethyl ether extracts (69.91 ± 1.03 mg/ml and 43.21 ± 0.14 mg/ml, respectively) are higher with respect to propyl gallate (IC_{50} 0.018 ± 0.04 mg/ml).

When the obtained data was evaluated according to the antioxidant activity of propyl gallate (IC_{50}: 0.09 ± 0.18 µg/ml), which was used as positive control in this study, the MeOH extracts were observed to have medium activity (IC_{50}: 89.43 ± 3.09 µg/ml), while the diethyl ether extract showed a weak activity (261.32 ± 2.72 µg/ml) in the TBA method.

The Apiaceae family plants have been used as food, spices and in traditional medicine. Many of these species have been used since ages around the world. The antioxidant effects of some Apiaceae species have been reported previously by several authors (12, 17-21). Previous studies have shown that different types of chemical constituents such as essential oil, phenolic acids, lignans, flavonoids, sterols, saponins and polyacetylenes were found in the various species of Apiaceae family (18, 19, 21-24). There are not many studies reporting antioxidant activities and active components of *B. radians* (5, 6). In this study the species was screened for antioxidant properties and total phenol content. The active components of the plant responsible for the antioxidant activity are currently unclear; therefore, bioassay-guided fractionation, isolation and characterization of the active constituents must be pursued in the future studies.

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