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Antioxidant activity and luteolin content of *Marchantia polymorpha* L.

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Abstract: Antioxidant activity of the methanol and ethyl acetate extracts of *Marchantia polymorpha* L., which grows naturally in Turkey, was tested by DPPH and ABTS methods. According to the applied methods, both extracts exhibited moderate activity. Some phenolic compounds that could be responsible for the activity were investigated by RP-HPLC, qualitatively and quantitatively, in the methanolic extract of liverwort. While caffeic acid, chlorogenic acid, rutin, myricetin, quercetin, and kaempferol were not found in the extract, luteolin as the liable antioxidant agent was determined as $0.0052 \pm 0.0002\%$ among the investigated compounds.

Key words: *Marchantia*, antioxidant activity, RP-HPLC, phenolic compounds, luteolin

Introduction

Liverworts and mosses are small, low-growing plants and constitute the phylum Bryophyta, which is phylogenetically placed between vascular plants and algae. Bryophyta has more than 20,000 members all over the world, and nearly 3000 bryophytes are reported to have medicinal value. The members of this unique division in the plant kingdom are now increasingly used as new sources of pharmaceuticals. One interesting class of bryophytes, the liverworts, possesses different therapeutic activities and has been therapeutically applied worldwide, especially in Indian culture. *Marchantia* species are among the important traditional Chinese medicinal herbs and are used in particular for the treatment of hepatitis and skin disorders owing to their antibiotic, anti-inflammatory, and diuretic properties (1,2). According to phytochemical studies, *Marchantia* species contain

terpenoids, flavonoids, steroids, and bis(bibenzyls) (2-6). *Marchantia polymorpha* L. (Marchantiaceae), a liverwort with a large thallus, is distributed all over the world and exhibits antimicrobial, anti-hepatic, antipyretic, and diuretic properties. It is also used to treat fractures, cuts, poisonous snake bites, burns, scalds, and open wounds (1,3,7). In previous studies, the enzyme polymorphism of European colonies of *M. polymorpha* was investigated for genetic variability, and the presence of 3 genetically distinct components was indicated (8). Due to the importance of the genetic variability of *M. polymorpha* and the absence of studies on Turkish samples, we chose to study this particular liverwort. To the best of our knowledge, the antioxidant activity and phenolic compound profile of *M. polymorpha* from Turkey is investigated for the first time in this study.

Materials and methods

Plant material

Marchantia polymorpha L. was collected from the Ilgaz region of Kastamonu Province in Anatolia in May 2007. The liverwort was identified by Prof. Dr. Barbaros Çetin, and the voucher specimen was deposited in the herbarium of the Ankara University Faculty of Pharmacy (AEF 25978).

Chemicals and standards

DPPH (D9132) and an ABTS antioxidant assay kit (CS0790) were purchased from Sigma (Germany). Chromatographic grade double-distilled water, HPLC grade methanol, acetonitrile, and analytical grade trifluoroacetic acid were used for HPLC analysis. The following phenolic compounds were purchased from Sigma (Germany): chlorogenic acid (C3878), caffeic acid (C0625), rutin (R5143), myricetin (M6760), quercetin (Q4951), luteolin (L9283), and kaempferol (K0133).

Extraction procedure

For antioxidant activity, 5 g of dried and powdered whole liverwort was extracted with methanol and ethyl acetate (100 mL each) by magnetic stirrer for 1 h (50 °C, 250 rpm). After filtration, the organic phases were evaporated completely by rotary evaporator (Buchi-R200), and the crude extracts were used in antioxidant activity tests.

For HPLC analysis, 200 mg of dried and powdered whole liverwort was extracted with methanol by magnetic stirrer for 6 h (50 °C, 250 rpm). The extract was then filtered and completed to 10.0 mL in a volumetric flask with methanol, passed through a 0.45 µm filter, and injected into the HPLC system.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The capacity to scavenge the “stable” free radical DPPH was monitored according to the modified method of Barros et al. (9). Various concentrations of methanol and ethyl acetate extracts (0.25 mL) were mixed with 2.75 mL of methanolic solution containing DPPH radical. The mixture was shaken vigorously and left to stand for 10 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption

at 517 nm. The radical scavenging activity (Inh%) was calculated as a percentage of DPPH discoloration using the equation: $\text{Inh}\% = [(A_{\text{DPPH}} - A_s)/A_{\text{DPPH}}] \times 100$, where A_s is the absorbance of the solution when the sample extract is added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Trolox (Sigma, Germany) was used as a standard.

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay. ABTS method was used to reinforce the results obtained from the DPPH radical scavenging activity assay. This assay was performed using an antioxidant assay kit supplied by Sigma with a modified Miller and Rice-Evans method (10). The principle of the antioxidant assay is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS to produce radical cation ABTS^+ , a soluble chromagen that is green and can be determined spectrophotometrically at 405 nm. Antioxidants suppress production of the radical cation in a concentration dependent manner, and the color intensity decreases proportionally. Trolox, a water soluble vitamin E analog, was used as a standard or control antioxidant. Myoglobin working solution (20 µL) and ABTS working solution (150 µL), which was prepared by mixing 3% hydrogen peroxide solution (25 µL) and ABTS solution (10 mL), were added to various concentrations of methanol and ethyl acetate extracts (10 µL). After an incubation period of 5 min, stop solution (100 µL) was added to the media, and endpoint absorbance values were recorded at 405 nm. The antioxidant activity (Inh%) was calculated as a percentage value by using the equation: $\text{Inh}\% = [(A_0 - A_s)/A_0] \times 100$, where A_s is the absorbance gained at the end of the process with sample extract, and A_0 is the absorbance of the control. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

HPLC analysis

The analysis was performed with a LC system consisting of an HP Agilent 1100 series quaternary pump, degasser, and photodiode array detector.

The samples were injected into an HP Agilent 1100 autosampler with thermostatted column compartment on a Phenomenex-Luna C18 column (5 μ , 250 mm; 4.6 mm) at 30 °C. The system was controlled, and data analysis was performed with Agilent Chem Station software. All calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas.

The analysis was performed by gradient elution with a flow rate of 1 mL/min. Column temperature was set to 30 °C. The mobile phase was a mixture of trifluoroacetic acid 0.1% in water (solution A), trifluoroacetic acid 0.1% in methanol (solution B), and trifluoroacetic acid 0.1% in acetonitrile (solution C). The composition of the gradient was (A:B:C); 80:10:10 at 0 min, 60:25:15 at 5 min, 50:30:20 at 10 min, 40:40:20 at 15 min, and 0:75:25 at 20 min. The duration between runs was 5 min. All solvents were filtered through a 0.45 μ m Millipore filter before use and degassed in an ultrasonic bath.

For quantification studies, luteolin (10 mg) was accurately weighed into a 10 mL volumetric flask, dissolved in the mobile phase, and filled up to volume to prepare stock solution. Standard solution was prepared in mobile phase at 5 different concentration levels in 10 mL volumetric flasks for the establishment of a calibration curve. Limit of detection (LOD) was established at a signal-to-noise ratio (S/N) of 3. Limit of quantification (LOQ) was established at a signal-to-noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by 6 injections of luteolin at the LOD and LOQ concentrations.

From each solution and sample 10 μ L were injected into the column, and the chromatograms

were recorded from 200 to 400 nm. Standard solutions were analyzed, and 3-dimensional chromatograms (wavelength, time, and absorbance) were obtained to select the optimum wavelength for detection of these phenolics with maximum sensitivity. Quantification was performed by measuring at 340 nm for luteolin using a photo-diode array detector. The chromatographic run time was 20 min and the column void volume was 1.60 min.

Results and discussion

The DPPH radical scavenging assay is a valid, easy, accurate, sensitive, and economical method commonly used to evaluate the ability of antioxidants to scavenge free radicals. On the other hand, ABTS assay is mainly based on inhibition of the absorbance of radical cation ABTS⁺ by antioxidants. Both methods are used often to determine the antioxidant profile of plant extracts and other sources (11-13).

In the current study DPPH and ABTS antioxidant activity methods were applied to evaluate the radical scavenging potential of the methanol and ethyl acetate extracts of *M. polymorpha*. According to DPPH radical scavenging activity results, the IC₅₀ value of the methanol extract of *M. polymorpha* was 0.4495 \pm 0.029 mg/mL, and the ethyl acetate extract of *M. polymorpha* was 0.2756 \pm 0.01 mg/mL, while Trolox exhibited an IC₅₀ of 0.0419 \pm 0.002 mg/mL. According to ABTS antioxidant activity results, the IC₅₀ value of the methanol extract of *M. polymorpha* was 0.2441 \pm 0.009 mg/mL, and the ethyl acetate extract of *M. polymorpha* was 0.2126 \pm 0.01 mg/mL, while Trolox exhibited an IC₅₀ value of 0.0431 \pm 0.001 mg/mL (Table).

Table. IC₅₀ values of *M. polymorpha* extracts.

	DPPH IC ₅₀ (mg/mL)	ABTS IC ₅₀ (mg/mL)
<i>M. polymorpha</i> methanol extract	0.4495 \pm 0.029	0.2441 \pm 0.009
<i>M. polymorpha</i> ethyl acetate extract	0.2756 \pm 0.01	0.2126 \pm 0.01
Trolox	0.0419 \pm 0.002	0.0431 \pm 0.001

Values are expressed as mean \pm SD.

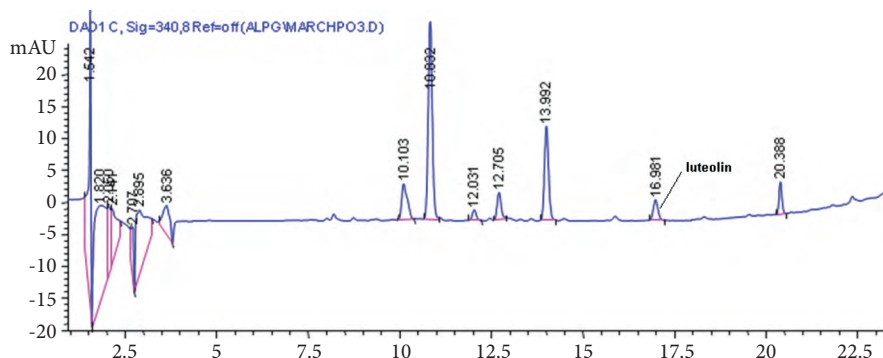


Figure. HPLC chromatogram of *M. polymorpha* methanol extract.

With regard to our antioxidant activity results and literature data on the phenolic compound profile of *Marchantia* species, we checked 7 phenolic compounds with known antioxidant activity (chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin, and kaempferol) in the extracts that could be responsible for the antioxidant potential of liverwort (14,15). According to qualitative RP-HPLC results, it was clear that among these phenolic compounds only luteolin was found in the methanol extract of liverwort (Figure). Retention times of these 7 phenolics were recorded as 6.5 min, 8.1 min, 10.3 min, 13.3 min, 16.5 min, 16.9 min, and 19.5 min, respectively.

The quantification of luteolin was done by external standard method and was $0.0052 \pm 0.0002\%$ (w/w). The linear relationship between peak areas and concentrations for luteolin can be expressed as $y = 40356x - 13.308$, with $r = 0.9999$. Retention time of luteolin was 16.9 min, LOD was $0.053 \mu\text{g/mL}$, and the LOQ was $0.177 \mu\text{g/mL}$ for luteolin. The precision of the method (within-day variations of replicate determinations) was checked by injecting luteolin 9 times at the LOQ level. The precision of the method, expressed as the RSD% at the LOQ level, was 3.082% for luteolin [RSD% = $(\text{SD}/\text{mean}) \times 100$]. In a previous study, the major flavonoids of *M. polymorpha* varieties were reported as apigenin and luteolin glucosides, and these were accompanied

by minor amounts of apigenin and luteolin (14). In another study that was performed on *M. convoluta*, quantification of quercetin, apigenin, and luteolin was carried out, and luteolin content was 0.0035% (15). These data support our findings on luteolin content of *M. polymorpha*.

In conclusion, the results of our study indicate that the Turkish liverwort *M. polymorpha* showed moderate activity by both antioxidant activity methods with close IC_{50} values compared to the results obtained with Trolox. Ethyl acetate extract seemed to have slightly higher antioxidant activity than methanol extract, although it did not contain any of the phenolic compounds investigated. Consequently, according to our HPLC results the antioxidant potential of the plant could be partially due to luteolin, but it is possible that other phenolics and bis(bibenzyls) in liverwort also contribute to the activity (16-18). The presence of these groups of compounds should be explored in future studies of Turkish liverworts.

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