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Investigation of the antioxidant properties of *Pterigynandrum filiforme* Hedw

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Abstract

The bryophytes, which constitute an important part of biodiversity, have a great interest in researches due to their secondary metabolites. The presence of secondary metabolites within the bryophytes is considered to be an indicator of the antioxidant capacity of these plant species. There are limited studies on the antioxidant content of bryophytes in Turkey. In this context, it is very important to investigate the antioxidant capacity of *Pterigynandrum filiforme* Hedw. plant species to determine its usability as a natural antioxidant. In the study, extraction of antioxidant compounds in the content of *Pterigynandrum filiforme* plant was carried out using ethanol solvent. DPPH (2,2-diphenyl-1-picrylhydrazole) radical removal activity, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical removal activity and reducing power antioxidant activity tests applied to the obtained extracts. As a result, *Pterigynandrum filiforme* has been found to be a natural antioxidant source. This situation strengthens the idea that bryophyte species may be an alternative natural antioxidant source for synthetic antioxidants.

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Introduction

Bryophytes are found all over the world except the seas. Bryophytes are taxonomically between algae and pteridophytes and they have approximately 24,000 species in the world. The most important role in the survival of these plants is the moisture of the environment. This plant has a wide distribution throughout the climates, from the tropical regions, where there is sufficient moisture, to subarctic regions (Abay and Kamer, 2010).

It is thought that bryophytes are not nutritious for humans. And until now, there is no reference to the fact that they are used as food for people. On the other side, various bryophytes species are widely used as a drug against burns, bruises, external scars, snake bites, pulmonary tuberculosis, neuralgia, fractures, convulsive boiling, uropathy, pneumonia, neurotensin in China (Garnier *et al.* 1969; Asakawa, 1999).

Bryophytes, which are at the bottom level of the food chain, involve many secondary metabolites. In favor of this advantage them, they have been used in treating traditional diseases.

Free radicals are atoms or molecules having one or more unpaired electrons in atomic or molecular orbitals. These unpaired electrons (s) impart great reactivity to the free radical. Free radicals are small molecules, have low activation energy and are short-lived. Their small size allows them to easily pass through the cell membranes (Jensen, 2003).

Oxidative stress is the imbalance between reactive oxygen species or other free radicals and the antioxidant system, and this imbalance can cause irreversible damage to the major parts of the cell.

The negative effects of oxidative stress on human health have become an important research topic. The imbalance between reactive oxygen species such as superoxide anion ($O^{2\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) caused by metabolic or external factors, and enzymatic or non-enzymatic antioxidant compounds causes oxidative stress.

Free oxygen radicals resulting from metabolic events may lead to detrimental effects such as cell aging, cardiovascular diseases, mutagenic changes and growth of cancerous tumors by attacking macromolecules such as DNA, protein, lipid and carbohydrates.

Antioxidants are molecules that have a phenolic function in their structure and which prevents the cell from being damaged by blocking the formation of free radicals or by sweeping the existing free radicals away (Kahkönen *et al.*, 1999). Antioxidants significantly inhibit or delay the oxidation of the substrate initiated with prooxidants (reactive oxygen and nitrogen species, free radicals) at lower concentrations than oxidizable substrates.

Prooxidants are toxic substances that cause oxidative damage in lipids, proteins and nucleic acids, resulting in various pathological events and/or diseases. The presence of these dangerous compounds makes antioxidants important for a healthy life (Cao and Prior, 1999). Because antioxidants effectively reduce the prooxidants and transform them into low toxicity or non-toxic products.

Bryophytes form part of the vegetation in the very humid climates of both temperate and tropical regions on the northern and southern slopes. They are found in the form of carpet in bright and green color in forest ecosystem and in peatlands on hummocks and in holes in green, brown and red colors. In addition, they can be found in nature on stone, on rock, in water and on rocks in the water as well as on the bodies and branches of the dead and living trees, on the decaying organic substances as semi-saprophytes and they can live in dry areas where humidity is minimal.

In our country, the studies related to bryophytes are generally for bryofloristic purposes and there are few studies about their antioxidant contents. This study, it is aimed to determine the antioxidant capacities of *Pterigynandrum filiforme* and to create a basis for further studies in this field.

Materials and methods

Reagents

Chemicals All chemicals used for analytical purposes were obtained from Sigma (St.Louis, MO).

Materials

It is a plant belonging to the Marchantiophyta Divison, Marchantiopsida class, Jubulales order, Pterigynandraceae family. It is the only taxon of the genus Pterigynandrum in our country (Ros *et al.*, 2013). *Pterigynandrum filiforme* is a reddish-brown plant of thin and medium-size and is a taxon spreading on tree barks and rocks and which mostly likes humid, shade and acidic environments (Dierβen, 2001; Smith, 2004). Research materials were collected from Örümcek Forests (Gümüşhane-Kürtün).

Henderson (1961), Turkey location of research in the grid system.

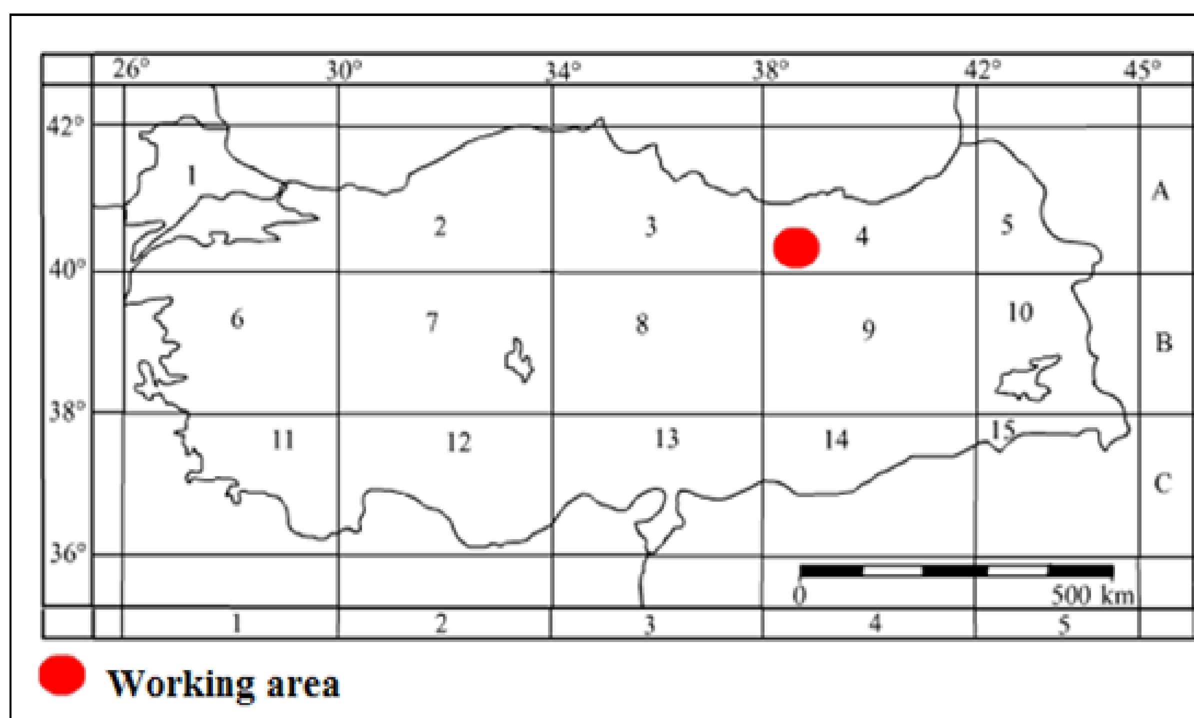

Fig. 1. Distribution: Turkey (A1, A2, A3, A4, B6, C11, C13).

Methods

Radical scavenging power

The radical scavenging power of *Pterigynandrum filiforme* was assessed by the method of Shimada *et al.* (1992) with slight modifications. The reaction mixture was a total volume of 3 ml, which included 2.9 ml of DPPH (1×10^{-4} M DPPH) and 0.1 ml of the corresponding sample at various concentrations. The solutions were left in the dark at room temperature for 30 min and the resulting colour was measured spectrophotometrically at 520 nm against blanks. Decreasing intensity of the color purple was related to a higher radical scavenging power percentage, which was calculated using the following equation; Radical scavenging power = $[1- (A_{S:30}/A_{B:30})]\times100$ where A_{S30} is the absorbance of sample and A_{B30} is the absorbance of blank at 30 min reaction time.

ABTS Determination of Radical Release Activity

$ABTS^{\bullet+}$ 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) measurement solution for radical scavenging activity (7 Mm), 30 mg of $ABTS^{\bullet+}$ radical cation prepared by dissolution in the 2.46 mM of $K_2S_2O_8$ aqueous solution (Re *et al.*, 1999). The ABTS measurement solution was diluted with water to an absorbance value of 7300 ± 0.005 at 734 nm. The required dilution procedure was applied on the extracts of *Pterigynandrum filiforme*. 2475 µL of $ABTS^{\bullet+}$ radical solution and 25 µL sample were added and incubated for 30 minutes. The absorbance

value at the wavelength of 734 nm was determined in the UV-Vis spectrophotometer after the incubation. Percent of the Radical Cation capacity was calculated from the absorbance values obtained.

The Radical Cation capacity (%) = 1-[Abs_{ref}÷Abs_{sample}] ×100

The results were expressed as mg Trolox® equivalent antioxidant capacity / gr sample.

Determination of reduction power

The measurement of reduction power was determined by making the necessary modifications in the method applied by Oyaizu (1988). After the necessary dilutions were applied on the extracts of *Pterigynandrum filiforme*, 50 µL of it was mixed rapidly with 375 µL ml 0.2 M phosphate buffer (pH 6.6) and 375 µL 1 % potassium ferricyanide solution. The mixture was left for incubation in a water bath at 50 °C for 20 minutes.

The reaction was terminated after the incubation by adding 375 µL of 10 % trichloroacetic acid (TCA) solution and it was centrifuged at 6,000 rpm for 10 minutes. 25 µL 0.1% $FeCl_3.6H_2O$ solution was added to 200 µL supernatant, which was separated after centrifugation. After incubation (5 min), the absorbance value of 700 nm wavelength was determined in the BioTek Eon C Microplate spectrophotometer device for the mixture, which was observed to have color formation. The results were expressed as mg Trolox® equivalent antioxidant capacity/gr sample.

The analyses were carried out in parallel with three different samples and the standard deviation was calculated with the arithmetic mean of the data.

Results and discussion

DPPH radical scavenging activity method is widely used to determine the antioxidant capacity of natural extracts. This method is based on the decrease of the absorbance at 520 nm as a result of the proton transfer reaction to the DPPH free radical due to the antioxidant effect. The antioxidant activity of a substance increases as the efficiency of its scavenging the free radicals in the environment feature increases (Tekeli *et al.*, 2008; Okan *et al.*, 2013).

Table 1. Station information.

Localities	Altitude (m)	Date	GPS Coordinates
1	1912	15-17.09.2017	N 40° 39' 53. 3" E 038° 59' 07. 7"

ABTS radical scavenging activity is based on the inhibition of the absorbance of ABTS radical cation by the antioxidant. The absorbance value of this green radical, which shows the maximum absorbance at 734 nm, decreases during the period of its reaction with the antioxidants. ABTS radical produced as a result of oxidation of potassium persulphate with ABTS in the experiments can be used in both lipophilic compounds and hydrophilic compounds (Orakçı, 2010; Okan *et al.*, 2013).

ABTS assay is practicable for both hydrophilic and lipophilic antioxidants. Both DPPH and ABTS methods are substrate-free. The popularity of these tests might be raised from simplicity and speed of analysis (Kazazic *et al.*, 2016).

The reducing capacity of the compounds can determine their antioxidant activity (Meir *et al.* 1995).

The reducing power increases depending on the absorbents as the concentration of the substance increases.

Reducing power is an indicator of the antioxidant effect. Antioxidant activities of the components were determined to the extent that ions in the environment could make the reduction. For this purpose, the absorbance changes at 700 nm in spectrophotometric terms for the color expansion in the blue-green colored complex were utilized. Our results show that DPPH radical scavenging, ABTS radical scavenging and reducing power activities are high (Table 2).

Table 2. DPPH, ABTS and reducing power values of *pterigynandrum filiforme*.

	DPPH	ABTS	İNDİRGEME GÜCÜ
	(mg trolax/g dry matter)	(mg trolax/g dry matter)	(mg trolax/g dry matter)
Pterigynandrum filiforme	0,1672±0,006	1,024±0,008	2,760±0,013

Owing to structural diversity, the antioxidant characteristics vary among the plants. Different antioxidant activity results can be linked to climate, plant species, testing methods and solvents used by the explorers (Kazazic *et al.*, 2016).

No literature on the antioxidant activity of *Pterigynandrum filiforme* was found in the literature search. As a result, *Pterigynandrum filiforme* plant has been found to be a natural antioxidant source. This situation strengthens the idea that bryophyte species may be a natural antioxidant source alternative to synthetic antioxidants.

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