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Antioxidant Properties of *Frullania Dilatata* (L.) Dumort

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Abstract: ***In the present study, crude extract of Frullania dilatata was prepared using ethanol. 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 activities were measured for the extract. Our results indicated that F. dilatata have potential antioxidant activity. These findings support the idea that bryophyte species may be a prospective natural antioxidant source.***

Keywords: Antioxidant, Bryophyte, Frullania dilatata, Oxidative stress, ROS

1. Introduction

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

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. On the other hand, antioxidants are molecules, which prevent the cell from being damaged by blocking formation of free radicals or by sweeping the existing free radicals away (Kahkönen *et al*., 1999). Moreover, 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.

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. Lower plants like Bryophytes form a part of the vegetation in the very humid climates of both temperate and tropical regions on the mountains. Bryophytes can survive under a wide variety of environmental conditions, such as growing forests, wetlands, dirt and rock habitats, etc. Moreover, they often form a significant part of ecosystems in spite of their small size and simple structure. 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 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. It is a plant belonging to the Marchantiophyta divison, Marchantiopsida class, Jubulales order, Frullaniaceae family. It is one of the 4 taxa of Frullania genus in our country (Özenoğlu Kiremit and Keçeli, 2009). *F. dilatata* 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).

In our country, the studies related to bryophytes are generally for bryofloristic purposes and there are few studies about their antioxidant contents. The present study aimed to determine the antioxidant capacities of *F. dilatata* and to create a basis for further studies in this field.

2. Materials and Methods

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

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Distribution: Turkey (A1, A2, A3, A4, A5, B6, C11, C12, C13) (Fig. 1).

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

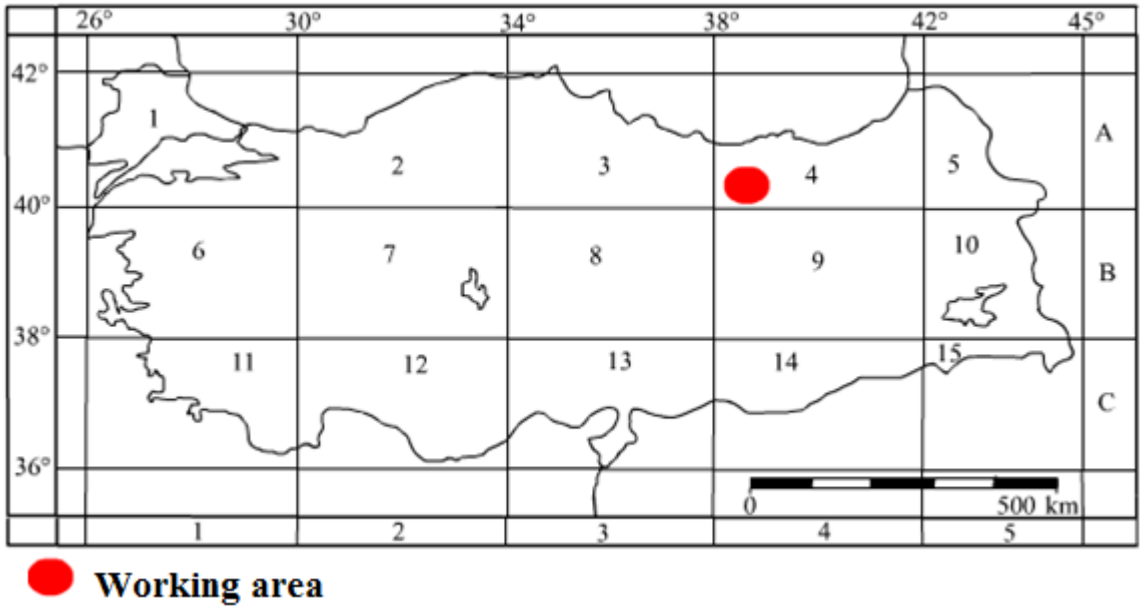

Figure 1: The map of working area

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

Trolox, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, Folin–Ciocalteu reagent, hydrochloric acid (HCl), iron (III) chloride hexahydrate, methanol, potassium ferricyanide, potassium peroxodisulfate, trichloroacetic acid (TCA), sodium hydroxide, sodium nitrite, sodium carbonate were purchased from Sigma-Aldrich. All chemicals used for analytical purposes were obtained from Sigma (St. Louis, MO). A Shimadzu 1601 model UV–Vis spectrophotometer (Tokyo, Japan) was used for antioxidant activity assays. Research materials were collected from Örümcek Forests (Kürtün, Gümüşhane, Turkey).

Radical scavenging power of *F. dilatata* were 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 then the absorbance was measured spectrophotometrically at 520 nm against blanks by a Shimadzu 1601 model UV–Vis spectrophotometer. A decreasing intensity of purple color was related to a higher percentage of free radical scavenging activity, which was calculated using the following equation;

Radical scavenging power = $[1- (A_{S:30}/A_{B:30})]\times100$ where, A_{S30} is absorbance of sample and A_{B30} is absorbance of blank at 30 min reaction time.

To measure the ABTS free radical scavenging activity, 30 mg of $ABTS^{\bullet+}$ radical cation solution was prepared by dissolution in 2.46 mM of $K_2S_2O_8$ aqueous solution (Re *et al*., 1999). The ABTS solution was then diluted with water to an absorbance value of 7300 ± 0.005 at 734 nm. The required dilution of the extracts of *F. dilatata*. 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 (%) = $1-[Abs_{ref} \div Abs_{sample}]$ x100

The results were expressed as mg Trolox® equivalent antioxidant capacity / g dry matter. The reducing power (RP) was determined by the modified method of Oyaizu (1988). 50 µL of *F. dilatata* extract was mixed 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 and was terminated by adding 375 µL 10% trichloroacetic acid (TCA) solution followed by centrifugation at 6000 rpm for 10 minutes. 25 µL 0.1% $FeCl_3.6H_2O$ solution was added to 200 µL supernatant which was separated after centrifugation. The absorbance of the solution was measured after incubation (5 min) at 700 nm wavelength by BioTek EONC Microplate spectrophotometer.

The results were expressed as mg Trolox® equivalent antioxidant capacity / g dry matter. The analyses were performed in triplicate and the standard deviation was calculated with the arithmetic mean of the data.

3. 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).

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 practible for both hydrophilic and lipophilic antioxidants. Both DPPH and ABTS methods are substrate-free. Popularity of these tests might be arised from simplicity and speed of analysis (Kazazic et al., 2016).

The reducing capacity of the compounds can determine its 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 antioxidant effect. Antioxidant activities of the components were determined to the extent that ions in the environment could make reduction. For this purpose, the absorbance changes at 700 nm in spectrophotometric terms for the colour expansion in the blue-green coloured complex were utilized.

Our results show that DPPH radical scavenging, ABTS radical scavenging and reducing power activities are high (Table 1).

Table 1: DPPH, ABTS and reducing power values of *Frullania dilatata*

	DPPH (mg trolax/g dry matter)	**ABTS** (mg trolax/g dry matter)	**Reducing power** (mg trolax/g dry matter)
Frullania dilatata	1.0734±0.062	3.196±0.021	9.790±0.253

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 *F. dilatata* was found in the literature search. As a result, *F. dilatata* plant has been found to be a natural antioxidant source. This situation strengthens the idea that bryophyte species may be natural antioxidant source alternative to synthetic antioxidants.

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