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DETERMINATION OF ANTIOXIDANT ACTIVITY AND DNA DAMAGE PROTECTION OF *MARCHANTIA POLYMORPHA* L.

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ABSTRACT

In this study, the potential biological effects of *Marchantia polymorpha* (Bryophyte) from Mount Ida (Canakkale/Turkey) were investigated. *M. polymorpha* was extracted using the Soxhlet apparatus with several organic solvents (hexane, chloroform, acetone, ethanol, methanol, and aqueous) at different concentrations. Phytochemical screening tests are done to detect certain compounds. The total phenolic content was determined with the Folin-Ciocalteau method. Total flavonoid content was determined with $AlCl_3$ method. Ethanolic extract was found to have higher total phenolic and flavonoid contents (18.24 GAE/g extract and 23.06 mgQE/g extract, respectively) than the other extracts. Antioxidant activities were examined by DPPH and ABTS radical scavenging assays. *M. polymorpha* extracts showed that dose-dependent percent inhibition. The aqueous extract had the highest DPPH percentage of scavenging activity (70.87% at a concentration of 80 µg/mL) followed by methanolic extract (68.59%). For ABTS radical scavenging activity, methanol extract showed the highest inhibition (73.12%) than other extracts. Various extracts were able to protect plasmid DNA by oxidative damage. The present study showed that *M. polymorpha* could be a potential source of antioxidants and protective effects against plasmid DNA.

KEYWORDS:
Antioxidant activity, DNA protection, *Marchantia polymorpha*, Ida Mountain (Canakkale, Turkey)

INTRODUCTION

Bryophytes are taxonomically placed between Thallophytes and Pteridophytes and subdivided into Bryophyta with 14,000 species, Marchantiophyta with almost 6000 species and Anthocerotophyta having 300 species. According to paleontological dating, they are the oldest terrestrial plant, originated 440-450 million years ago [1]. The studies have also reported that bryophytes has lots of medical properties such as anti-pyretic, anti-arthritic, anti-hematemesis, anti-pneumonia, anti-tuberculosis, anti-phlogistic, anti-hepatic, anti-dermatomycosis, and wound-burn-fracture healing. Among the bryophytes, especially in the liverworts (Marchantiophyta), components such as lipophilic mono-, sesqui-, and diterpenoid compounds have been found. The biologically active components of Marchantiophyta are fragrant, bitterness, sharpness, and sweetness, as well as allergic contact dermatitis, heart-strengthening, muscle relaxant, plant growth regulator, superoxide release inhibitor, thromboxane synthesis inhibitor, vasopressin activity counter, insecticidal. It has oxygenase inhibitor, antimicrobial, antifungal, and cytotoxic effects [2].

Liverworts contain various terpenes, terpenoids, phenolic compounds, bibenzyls and bisbenzyls. These substances have metabolic activities against some other living forms. Terpenoids and bis[bibenzyls] from *Marchantia polymorpha* L. have been known for antimicrobial activities [3-4]. Marchantin A, marchantin B, marchantin E, neomarchantin A, plagiochin E, riccardin H, 13,13'-O-isoproylidenericcardin D shows antifungal activity [5]. Antiparasitic activity of Marchantin A and Marchantin E is known [6]. Marchantine A, Marchantin E, plagiochin A marked to have antiviral activity against Influenza A and B viruses [7]. Macrocyclic bis [bi-benzyls] are found mainly in liverworts and lichens, especially in Marchantiaceae, Radulaceae, Aneuraceae, Plagiochilaceae, Aytoniaceae, Jungermanniaceae, and Ricciaceae of liverworts. Marchantin A, a representative bis [bibenzyl]-type constituent, is widespread in *Marchantia* species.

Antioxidants are defined as substances that prevent or delay the oxidation of substances that can easily oxidize. Thus, it protects a substance from oxidizing. Free radicals that enter the organism from the outside or produced in the organism act as an oxidant, preventing the organism from remaining healthy. Free radicals; maybe in the form of oxygen derivatives or nitrogen derivatives. In a normal cell, oxidants and antioxidants are in balance. Disruption of this balance in favor of free oxygen radicals is called oxidative stress. Oxidative stress can cause very serious health problems, including cell death.

Therefore, antioxidants stand out as very important molecules in order not to disturb the oxidant-antioxidant balance. The importance of antioxidants has led researchers to determine the antioxidant capacities of foods and biological samples and thus to develop antioxidant capacity methods.

In this study, the total antioxidant capacity of liverwort, *M. polymorpha*, determined by DPPH and ABTS methods and the results compared. The Folin-Ciocalteu method used for the total amount of phenolic substances.

MATERIALS AND METHODS

Plant materials. Gametophytes of *M. polymorpha* L. (Figure 1) were collected from Ida Mountain (Kazdag) (03.05.2018), Canakkale, Turkey. The plant was identified by Prof. Dr. Ozlem Tonguc Yayintas. Voucher specimens are kept in the Canakkale Onsekiz Mart University, School of Applied Sciences.

Preparation of plant extracts. The dried and powdered *M. polymorpha* was extracted from non-polar to polar solvents using soxhlet apparatus (Wisd. Wise Therm). After filtered through a filter paper, the solvents were evaporated using a rotary evaporator to obtain crude extracts.

Chemicals and reagents. 2,2-Diphenyl-1-picrilhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Butylated hydroxytoluene (BHT), Quercetin, Gallic acid, Folin-Ciocalteau, potassium persulfate ($K_2S_2O_8$), agarose, tris, EDTA, H_2O_2 were purchased from Sigma-Aldrich. pBR322 plasmid DNA was purchased from Thermo Fisher Scientific. All the solvents were purchased by Merck.

Phytochemical screening. Phytochemical screening of the *M. polymorpha* extracts was done with standard protocols to determine coumarins, cardiac glycosides, phlabotannins, quinones, flavanones, anthocyanins, tannins, and saponins [8-9].

Determination of total phenolic content. Total phenolic content was determined using the Folin-Ciocalteau reagent according to the methods of [10]. A calibration curve was prepared using Gallic acid as standard phenolic compound. The absorbance was recorded by spectrophotometer at 760 nm. The total phenolic content was expressed as mg gallic acid equivalents/g extract.

Determination of total flavonoid content. The total amount of flavonoid content was determined by Matejić et al. [11]. A calibration curve was prepared using quercetin as standard flavonoid compound. The absorbance value of the mixture was measured at 415 nm with an UV-Vis spectrophotometer. The total amount of flavonoid content was expressed in mg quercetin/g extract.

DPPH Radical Scavenging Assay. 2,2-Diphenyl-1-picrilhydrazyl **(**DPPH) radical scavenging assay of the *M. polymorpha* extracts was measured by the method described by Brand-Williams et al. with a slight modification [12]. Different concentrations of extracts and standard were mixed with methanolic DPPH solution. Then the mixture was shaken vigorously and allowed to stand for 30 min. in the dark at room temperature. The reduction in absorption was recorded at 517 nm using an ultraviolet-visible (UV-Vis) spectrophotometer against methanol as a blank. The experiment was carried out in triplicate at each concentration and BHT used as a standard. DPPH free radical scavenging activity of the extracts (%) was calculated using the formula [(Absc-Abst)/Absc] x100, where Absc is the absorbance of the control and Abst is the absorbance of the samples and standard.

ABTS Radical Scavenging Assay. 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate (ABTS) radical assay was determined according to the method of Re et al. [13]. The assay generated by the reaction of ABTS solution (7mM) in water and potassium persulfate (2.45 mM) for 12-16 h in the dark at room temperature. Various concentration of the extracts was treated with diluted ABTS solution then the mixture was incubating in dark at room temperature for 30 min. The absorbance of the solution was measured spectrophotometrically at 734 nm. All the analyses were performed in triplicate. ABTS scavenging activity (%) was calculated using the following formula: $[(Abs_0-Abs_1)/Abs_0]$ x100, where Absc is the absorbance of the control and Abst is the absorbance of the extract/standard.

DNA damage protection potential. DNA damage protection potential was performed using supercoiled pBR322 plasmid DNA by agarose gel electrophoresis with an oxidizing agent. pBR322 DNA in Tris-HCl buffer (10 mM, pH:7.2) treated with the extracts of *M. polymorpha* at 37^0C for 3h. To determine the mechanism of damage protection potential 3% H_2O_2 was added to mixture as an oxidizing agent. After incubation loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol) was added and samples were analyzed by gel electrophoresis on 1% agarose gel (containing ethidium bromide) in TBE buffer (40 mM Tris-acetate, 1mM EDTA at pH 8.2) for 1 hr. at 60V. Then, the DNA bands were visualized under UV light and photographed (Quantum ST4 gel imagining system, Vilbar Lourmat).

TABLE 1
Phytochemical screening of *Marchantia polymorpha*

Cited References	HE	CE	AE	EE	ME	AQE	Compound
Current Work	+	+++	+	+++	+++	++	
[23]	+	+	NULL	+	+	-	Coumarins
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	+	++	+	++	++	+++	
[23]	NULL	NULL	NULL	NULL	NULL	NULL	Cardiac
[25]	NULL	NULL	NULL	NULL	NULL	NULL	Glycosides
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	-	-	-	-	-	+	
[23]	NULL	NULL	NULL	NULL	NULL	NULL	Phlabotan-
[25]	NULL	NULL	NULL	NULL	NULL	NULL	nis
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	++	+++	++	++	+++	-	
[23]	-	-	NULL	+++	+++	++	Flavanones
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	-	-	-	-	-	-	
[23]	NULL	NULL	NULL	NULL	NULL	NULL	Anthocya-
[25]	NULL	NULL	NULL	NULL	NULL	NULL	nins
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	-	+	-	++	-	-	
[23]	-	+	NULL	+	+	-	Tannins
[22]	NULL	NULL	NULL	NULL	NULL	NULL	
Current Work	-	-	-	-	-	-	
[23]	-	-	NULL	+	+	+	Saponins
[22]	NULL	NULL	NULL	NULL	NULL	NULL	

HE: Hexane extract; **CE:** Chloroform extract; **AE:** Acetone extract; **EE:** Ethanol extract; **ME:** Methanol extract; **AQE:** Aqueous extract; **+++:** Highly present, **++:** Moderately, **+:** Low, **-:** Absent

RESULTS

The majority of plants show antimicrobial activity thanks to the phytochemicals they contain [14]. Metabolites that cause antimicrobial activity in moss are primary metabolites. It is mandatory for almost all living organisms and derives from primary metabolism reactions. These include carbohydrates, nucleotides, proteins, tricarboxylic acid cycle intermediates, lipids, diffuse pigments of photosynthetic processes, and lignin. The primary metabolism of bryophytes is very similar to that of vascular plants. Essential compounds such as cellulose in the cell walls include chlorophyll a, chlorophyll b, main carotenoids, starch, nucleic acids, sugars, and some lipids. Plants produce organic substances that do not have vital value with their secondary metabolism and are not directly involved in the growth and development of the plant. Secondary metabolites chemically collected in 3 different groups. These are terpenes, phenolic, and alkaloids [2]. In studies with bryophytes, lignin-like aromatic compounds, carbohydrates, amino acids encountered.

Phytochemical screening. Phenolic substances are found in fruits, vegetables, leaves, seeds, flowers, and peels. In addition to being a part of human nutrition by being consumed naturally, they also used for medical purposes. Since ancient times, people have been making use of herbal resources to improve common health problems. However, the importance of these compounds on health has been understood by scientific studies in recent years. Phenolic compounds can contribute to the taste and aroma of many plant-based foods [15].

The phytochemical screening is showed in Table 1. It indicated the presence of secondary metabolites like alkaloids, phenols, tannins, flavonoids, coumarins, steroids, and sugars, whereas anthocyanin's and saponins were not present.

Total phenolic content. Phenolic compounds, which have attracted attention in the last decade, are thought to trigger pharmacological effects on diet through antioxidant mechanisms. In addition to the antioxidant effects of natural phenolic compounds, it is understood that the therapeutic potential of blood sugar should be investigated.

Total phenolic content of various extracts of *M. polymorpha* was varying widely between 4.54 ± 0.07 to 18.24±0.20 GAE/g extract. Ethanolic extract of *M. polymorpha* was demonstrating higher total phenolic content (18.24±0.20 GAE/g extract) than the

methanol, chloroform, acetone, hexane, and aqueous solvent extracts; 13.68±0.11; 12.68±0.18; 10.26 ±0.08; 8.32±0.15; and 4.54±0.07, respectively. In a previous study on *M. polymorpha*, one of the phenolic acids, Rosmarinic acid, was found to be higher than other acids (Table 2). This is a highly valued natural phenolic compound that is very commonly found in plants of the Lamiaceae and Boraginaceae families. It is also found in other members of higher plant families and in some of fern and horned liverwort species. Rosmarinic acid has been reported to have some biological activities such as anti-HIV-1, antibacterial, antioxidant, anti-carcinogen, anti-allergic activities and antiviral properties in *in-vitro* studies. In vivo studies have also shown that Rosmarinic acid has antiallergic, antithrombosis, and anticarcinogenic properties. The industry is increasingly interested in natural food preservatives, antimicrobials, and antioxidants because of their many beneficial effects [16].

Total flavonoid content. The total flavonoid content was high in ethanol (23.06 ± 0.06 mg QE/g extract) followed by chloroform and methanol (16.05 ± 0.13 and 15.23 ± 0.17 mg QE/g extract, respectively). Some of the studies also shown that the total flavonoid contents of liverworts were mostly higher than mosses. The total flavonoid contents of epiphytic bryophytes and growing at lower light levels and at low-latitudes species of bryophytes were higher than other bryophytes [17].

Antioxidant activity. DPPH free radical scavenging activity. Antioxidant substances are strong enough to remove free radicals in the environment. DPPH* and ABTS+ are stable free radical method which is a rapid, easy and sensitive way to study the antioxidant activity of plant extracts [18]. The % inhibition values of DPPH free radical removal activity depending on the absorbance's measured at 517 nm in the radical removal experiments performed according to the DPPH method shown in Figure 1.

TABLE 2
Phenolic acid content of *M. polymorpha* [22]

Compound Number	Compound Name	Retention Time	Area	Area%	Height	Calculated Quantity
1	Gallic Acid	4.941	37.579	12.24	3449	0.090
2	4-Hydroxy benzoic acid	13.758	9608	3.13	1153	0.047
3	Chlorogenic Acid	14.112	6985	2.28	551	0.031
4	Vanillic Acid	16.127	8522	2.78	856	0.036
5	Caffeic Acid	16.644	5427	1.77	505	0.012
6	Syrinic Acid	17.304	3191	1.04	354	0.008
7	Kumaric Acid	23.839	3920	1.28	336	0.006
8	Routine	28.024	1333	0.43	122	0.012
9	Benzoic Acid	31.129	871	0.28	124	0.015
10	Cinnamic Acid	34.058	1349	0.44	100	0.002
11	**Rosmarinic Acid**	**37.031**	**224521**	**73.14**	**20076**	**1.199**
12	Quarcetin	42.742	3674	1.20	609	0.190

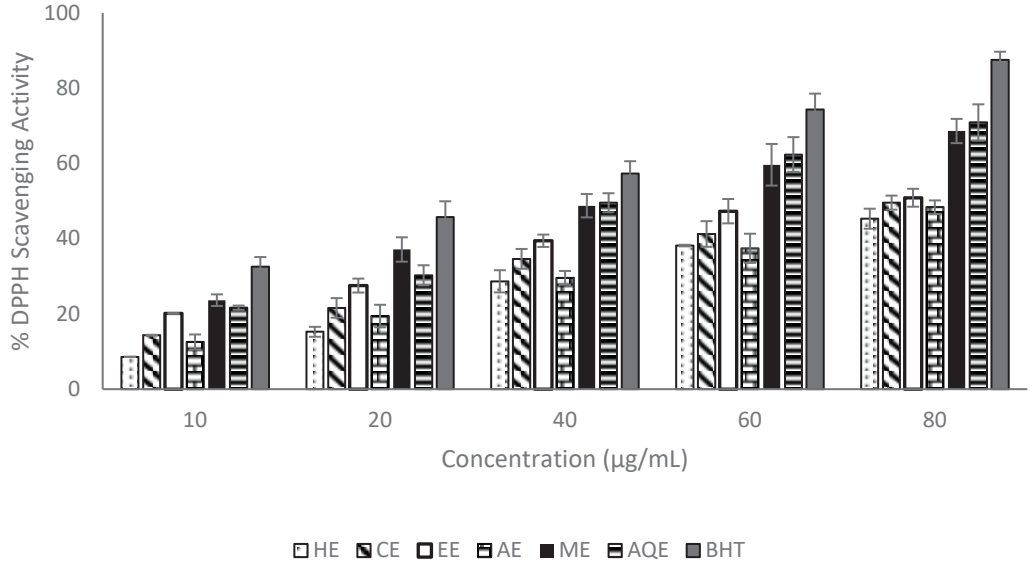

FIGURE 1
DPPH free radical scavenging activity of *M. polymorpha* extracts.

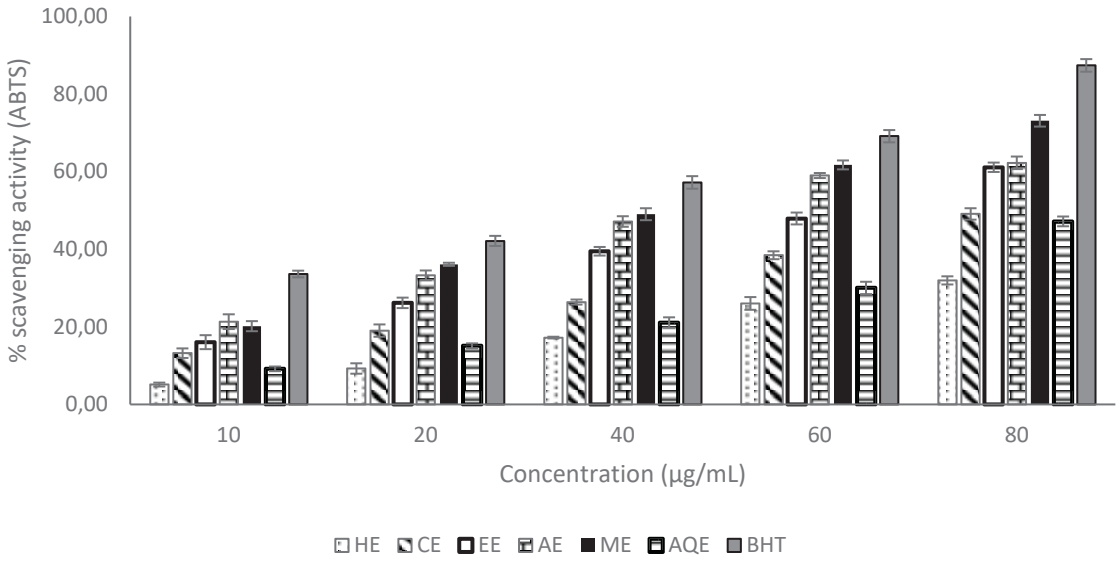

FIGURE 2
ABTS radical scavenging activity of *M. polymorpha* extracts.

Different concentrations of hexane, chloroform, acetone, ethanol, methanol, and aqueous extracts of *M. polymorpha* for free radicals of DPPH showed remarkable scavenging activities. Aqueous extract showed the highest percentage of scavenging activity (lowest IC_{50} 3.24 µg/ml) followed by methanol extract.

ABTS radical scavenging activity. ABTS+ (2, 2 azobis-(3-ethylben-zothiozoline-6-sulphonic acid)) radical cation scavenging capacities of hexane, chloroform, acetone, ethanol, methanol, aqueous extracts including BHT were arranged in Figure 2. Among various fractions methanol extract possessed the highest ABTS radical scavenging activity (73.12%), while hexane showed the lowest ABTS radical scavenging activity (31.97 %).

Effect of Reactive Oxygen Species on DNA. Many factors can cause oxidative damage in the DNA structure. High oxygen concentration, ionizing radiation, xanthine oxidase, and various chemicals cause excessive radicals to form, causing direct damage to DNA. For example, it can affect DNA, causing cell change, denaturation, and even fatal outcomes [19-20].

In the presence of H_2O_2 as the oxidizer, DNA damage protection results were examined from images of bands on agarose gel. When the original supercoiled form (Form I) of the pBR322 plasmid DNA is opened with damage, the open circular form (Form II) occurs, and with more fractures, the linear form (Form III) can also be found. When DNA undergoes gel electrophoresis, Form I progress faster in gel than others, while Form II moves slower and Form III, moves between Form I and Form II.

The hexane and acetone extracts cleaved the DNA and forming a Form II, aqueous extract cleaved the DNA at a concentration of 2 µM forming Form III and Form II structure. In the presence of chloroform, ethanolic and methanolic extracts were able to protect supercoiled pBR322 DNA against by the oxidative damage (Figure 3).

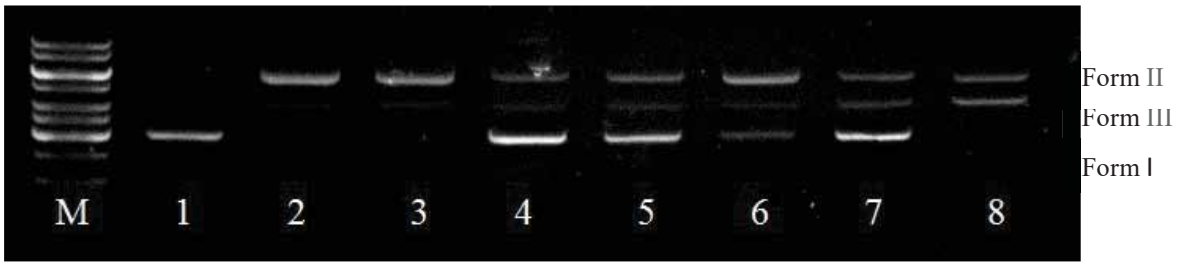

FIGURE 3
Agarose gel electrophoresis patterns for the oxidative damage of pBR322 DNA by *M. polymorpha* extracts
M. Marker, 1. Plazmid DNA, 2. DNA+ H_2O_2, 3. DNA+ 2 µM HE + H_2O_2, 4. DNA+ 2 µM CE+ H_2O_2, 5. DNA+ 2 µM EE+ H_2O_2, 6. DNA+ 2 µM AE+ H_2O_2, 7. DNA+ 2 µM ME+ H_2O_2, 8. DNA+ 2 µM AQE+H_2O_2

DISCUSSION

Secondary compounds of liverworts such as terpenoids, aromatic compounds, acetogenins, coumarins, cardiac glycosides, phlabotannins, quinones, flavanones, anthocyanins, tannins, and saponins several of which show interesting biological activity [21, 22]. The amount of total phenol content reported in ethanolic extract in the present study was higher (18.24 mg/g) than the Krishnan and Murugan (2013) studies (12.5 mg/g) [23]. When we compared our studies to Rana et al (2018), ethanol extract (19.31 mg/g) shown that notable [24]. The quantity of flavonoid value found the highest in ethanol extract (23.06 mg/g). Ethanol solvent indicated the highest efficiency for flavonoids and phenolic compounds. This situation can be explaining spring period data higher than winter period because of climatic changes and plant growth factors. Most of the literature mentions that on total flavonoid concentrations in Spermatophyte species, the range was from 0.095 mg/g to 25.01 mg/g and in most Pteridophytes was greater than 50.0 mg/g [17].

The screening for the antioxidant property by DPPH assay revealed slightly higher antioxidant activity in aqueous extract of *M. polymorpha* than methanolic extract. Remarkably, *M. polymorpha* revealed considerable DPPH radical scavenging activities comparable with higher plants and also with the reference compounds. This finding sign evaluated *M. polymorpha* as potential radical scavengers which is ideal natural antioxidants. The antioxidant property by ABTS assay revealed higher antioxidant activity in methanolic extract of *M. polymorpha* than acetone and ethanolic extracts. Krishnan and Murugan (2013) reported that inhibition of concentration at 1000 µg/mL ethanol and aqueous extract of the *M. polymorpha* 88% and 67.21%, respectively [23].

CONCLUSION

Flora of Turkey, the number of taxa rich in terms of vascular plants and has a characteristic that attracts attention with a high rate of endemism. The bryophytes of our country are also the group of plants that contain the most biodiversity in our flora after seed plants. With this study, physical and chemical properties, antioxidant activity and phenolic profile contents of bryophytes, which are not well known and accepted as primitive plant group, were determined. It is thought that determining the variety and amount of these plants that grow naturally in many parts of our country and increasing the evaluation possibilities will be very beneficial for the economic, social, and pharmaceutical industry. The possibility of *M. polymorpha*, whose antioxidant properties have been proven, used as a drug active substance, as a subject of a later project, it is aimed to investigate its synthesis ability, ability to be used as a drug substance and preservative.

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