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Antioxidant, Antimicrobial and Some Chemical Composition of *Plagiochila asplenioides* (L.) Dumort Extract

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Abstract

Bryophytes are the oldest terrestrial plants in the world, about 3.000 taxon are known to have medicinal properties. *Plagiochila asplenioides* (L.) Dumort, which is one kind of bryophytes, was examined in this study. We investigated the antimicrobial, antioxidant, fatty acid, mineral analysis, and some chemical properties of the extracts obtained from the bryophyte. It was determined that the *P. asplenioides* extract had a moderate effect in terms of antioxidant properties and total phenolic content. In terms of fatty acid compositions, oleic and palmitic acid were found to be high. As a result of the mineral analysis, it has been observed that it can accumulate high levels of potassium (K), calcium (Ca), iron (Fe) and aluminum (Al). In addition, it was observed that the extracts obtained from *P. asplenioides* showed only a moderate inhibitory effect against *Escherichia coli* and *Salmonella typhimurium.*

Keywords: Antioxidant, bryophyte, chemical analysis, mineral analysis, *Plagiochila asplenioides.*

Plagiochila asplenioides (L.) Dumort Ekstraktlarının Antioksidan, Antimikrobiyal ve Bazı Kimyasal Bileşimi

Öz

Briyofitler dünyanın en eski karasal bitkileridir, yaklaşık 3000 taksonun tıbbi özelliğe sahip oldukları bilinmektedir. Bu çalışmada, bir briyofit türü olan *Plagiochila asplenioides* (L) Dumort incelenmiştir. Bu briyofitten elde edilen ekstraktların, antimikrobiyal, antioksidan, yağ asitleri, mineral analilzeri ve bazı kimyasal özellikleri araştırılmıştır. Antioksidan özellikleri ve toplam fenolik içeriği bakımından *P. asplenioides* ekstraktlarının ılımlı bir etki gösterdiği tespit edilmiştir. Yağ asidi kompozisyonları bakımından ise oleik ve palmitik asit yüksek oranda bulunmuştur. Yapılan mineral analizleri sonucunda yüksek oranda potasyum (K), kalsiyum (Ca), demir (Fe) ve alüminyüm (Al) biriktirebildiği görülmüştür. Ayrıca *P. asplenioides*'den elde edilen ekstraktların sadece *Escherichia coli* ve *Salmonella typhimurium* karşı ılımlı bir inhibisyon etkisi gösterdiği gözlemlenmiştir.

Anahtar kelimeler: Antioksidan, briyofit, kimyasal analiz, mineral analiz, *Plagiochila asplenioides.*

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1.Introduction

From the evolution theory point of view, bryophytes are considered among the oldest terrestrial plants (probably the first true plants) directly placed between algae and vascular plants (Gofinet and Shaw, 2008; Glime, 2017). Bryophytes, with more than 20.000 species, are further divided into three main categories: Bryophyta (mosses ~14.000 species), Marchantiophyta (liverworts, ~6000 species) and Anthocerotophyta (hornworts ~300 species) can be found everywhere (except in the sea) in the world (Glime, 2017). In this regard, bryophytes are very important plant for forest ecosystem. Studies of bryophyte chemistry has been very limited, although its spread over such a wide geography on the earth (Klavina et al., 2012). Klavina (2018) reported that chemical analysis of only 2% of known mosses and 6% of liverworts have been realized (Klavina, 2018). According to some researchers, this is because of the fact that bryophytes are not appropriate in human nutrition (Asakawa et al., 2013; Commisso et al., 2021). Nevertheless some attention has been dedicated to secondary metabolite research on bryophytes in recent years, because they have been widely used as medicinal plants in various parts of the world (particularly in China) for various ilnesses (Asakawa et al., 2013; Vollár et al., 2018). Some researchers stated that nearly 3000 taxon of bryophytes have been medicinal importance (Türker and Ünal, 2020; Manisara et al., 2021).

Around the world, especially in developed countries as well as Turkey, there has been an increasing in interest in high antioxidant plant (Okan et al., 2019). Bryophytes contain valuable seconder metabolite such as terpenoids, flavanoids and bibenzyls (Marko et al., 2001). The main benefit of these compounds is possess their antioxidant properties. At first glance it is hard to imagine antioxidant properties of bryophytes compared to higher plants, but if examining deeper, it looks clear, bryophytes synthetize unique compounds that have shown a wide range of biological activities not only such as antioxidant, antifungal, antimicrobial but also antiviral, anticarcinogenic, muscle relaxing, anti-obesity activities, insecticidal, neurotrophic and cardiotonic (Cianciullo et al., 2022). Furthermore, it is known that the antioxidant capacity of mosses is higher than some higher plants (Türker and Ünal, 2020). In bryophytes, highly unsaturated fatty acids may also responsible role as an antioxidant in the human body (Ichikawa et al., 1983; Tedone et al., 2011).

As already mentioned, most of bryophyte species are shown antioxidant activity as well as antimicrobial activity. Studies show that carbohydrate from mosses in bogs react with undergo acidic and anaerobic condition. As a consequence of this reaction creation antimicrobial condition via Maillard and this is leads to conservation of organic, fat, and aminoacids containing materials (Klavina, 2018). Alcoholic and the aqueous extract of around 150 different species bryophyte containing flavonoids have been reported to show antimicrobial effects against various group of fungi as well as gram negative and gram positive bacteria (Mishra et al., 2014).

In light of this information, the present study focused on the antioxidant, antimicrobial, some nutritional properties and chemical composition of *P. asplenioides* which is a bryophyte species. When the literature is examined, it is not find that chemical composition, fatty acid, antioxidant and antimicrobial study by *P. asplenioides*. Therefore this study can be first study in this respect.

2. Materials and Methods

2.1. Plant Material

P. asplenioides was collected in the growing season of 2022 in Trabzon. The plants were identified and storage in the Herbarium at the Department of Biology, Faculty of Science, Karadeniz Technical University. It was carefully cleaned and washed by using distilled water to remove soil and other contaminants. Finally, samples were dried at room temperature under the shade.

2.2. Extraction process of the *P. asplenioides*

Approximately 2.5 g sample was added to 50 mL 99% methanol and homogenized in a blender for 5 minutes. The mixture was transferred to Falcon tube. All tubes were continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. The particles were then filtered with filter paper (Whatman) and concentrated in a rotary evaporator (IKA-Werke,Staufen, Germany) at 40 °C. The residue was disolved in methanol to a known final concentration and kept at 4 °C until.

2.3. Antioxidant activity analysis

UV-1800 (Shimadzu, Japan) spectrophotometer was used to measure absorbances in all antioxidant analysis.

2.3.1. Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of *P. asplenioides* extracts was spechtrophotometrically determined by the phosphomlybdenum assay using

the method described by literature (Prieto et al., 1999). According to this method, added 2500 µL deionized water in 500 µL extract. The solution was mixed with 1000 µL phosphomolybdenum reagent (28 mM monobasic sodiumphospate and 4mM Ammonium heptamolybdate tetrahydrate in 0.6 M sulphuric acid) in capped test tubes. The mixture was vortex after that sample in capped test tube was incubated for 90 min in a water bath 95 °C. The absorbance of 695 nm was measured after incubation at room temperature. Ascorbic acid was used as a standard substance.

2.3.2. Free radical scavenging activity (DPPH)

DPPH scavenging activity was measured according to method described by Brand-Williams et al., (1995). Extract solution (100 µL each) were mixed with 3000 µL freshly prepared 10 mM DPPH solution disolved in methanol. The mixture was shaken and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. The absorbance was read at 517 nm against a control using a spectrophotometer. The same concentration of extracts without DPPH was used a blank. The values were shown as ascorbic acid mg/kg

2.3.3. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was conducted as described by literature (Benzie and Strain, 1996) For FRAP activity assay of plant extract, fresh FRAP reagent was made by adding 300 mM sodium acetate buffer solution (pH: 3.6), 10 mM aqueous TPTZ solutions in 40 mM HCl, and 20 mM aqueous $FeCl_3$ solution in a ratio 10:1:1. In brief, 250 µL extract solution was added on 2750 µL of freshly prepared FRAP reagent. The mixture was incubated at 37 °C for 15 min before using a spectrophotometer. The absorbance was measured at 593 nm against a control. Results were expressed as mg $FeSO_4$/ 100 g.

2.3.4. ABTS $^{\bullet+}$ assay protocol

The ABTS+ radical cation stock solution was consisting of 7 mM aqueous ABTS and 2.45 mM potassium persulfate solution (1/1, v/v) for 120 minute in a darkness at room temperature. Next, it was diluted in methanol until an absorbance of 0.700 ± 0.020 at 734 nm and room temperature to obtain the ABTS+ working solution. The extract (150 µL) and ABTS solution (2850 µL) mix were vortexed and transferred to the spectrophotometer cuvettes. The absorbance was read at 734 nm against a control (Pellegrini et al., 2003). Trolox standard curve to exptess the results as mg Trolox/kg.

2.3.5. Total Phenolic Content (TPC)

Total phenolic contents of the plant extracts were determined by Folin-Ciocalteu reagent (Kasangana et al., 2015). Dry extracts (300 µL) were solubilized with methanol and combined with 200 µL 2 N Folin-Ciocalteu reagents and 600 µL of 10% Na_2CO_3. The mixture was vortex and incubated 120 min in darkness at room temperature. After that, the absorbance of the mixture was measured at 760 nm using a spectrophotometer. Gallic acid was used to prepare a calibration curve and results are expressed in gallic acid equivalents (mg GAE/g dry extract).

2.4. Total Flavanoid Analysis

The TFC of *P. asplenioides* extract was evaluated according to the method described by Fukumoto and Mazza (2000) with some modifications. Briefly, 500 µL extract was dissolved 3200 µL methanol (% 30 v/v). 150 µL of 0.5 M $NaNO_2$ and 150 µL of 0.3 M $AlCl_3$ were added to a test tube. Also, 1 ml of 1 M NaOH was added after five minutes on same test tube and incubated at room temperature for 10 minutes. Then, the absorbance was measured against a blank 506 nm. Results were expressed as mg quercetin equivalents (QE) per kg (mg QE/ g).

2.5. Determination of Mineral Analysis

Mineral contents of *P. asplenioides* was determined by MP-AES. Prior to mineral analysis, plant sample was digested in a closed microwave digestion system (Milestone Start D model) using appropriate solvent mixtures. For that purpose 0.500 g plant material was placed in Teflon vessels, then 6 mL HNO_3 and 2 mL H_2O_2 were added into the vessels. The temperature was increased gradually, starting from 50 °C and increasing up to 200 °C. The mixture was left to cool down and digested samples were transferred to 100 mL volumetric flask. The volume of the contents were made to 50 mL with distilled water. It was prepared µg/ml solutions from 1000 ppm solutions of Fe, Ca, Zn, Cd, Mg, Cu, Co, Ni, Al, Mn, Pb, Cr, K metal ions. The sample was filtered with 0.45 micron cellulose filter before mineral determination. (NMKL 170 and NMKL 161).

2.6. Determintaion of total lipid, protein and ash contents

For lipid content, five grams of ground sample were placed in cellulose extraction catridge which was chapped on with cotton wool. The catridge was then put in the soxhlet chamber and this was placed into thermostated water bath at 60 °C and fitted to a tared distilation flask containing 100 mL of n-Hexane and 2-3 boiling glass refulator. After extraction 16 h, the largest part of solvent was

released by rotary-evaporator. The residue was transferred to a 103 °C oven. The sample was kept until constant weight in oven. The amount of total oil content was calculated as percantage accordint to the formula.

Total Lipid Content (%):$\frac{m1}{m}$ x100
m: Mass of sample in gramme
m1:Mass of dry extract in gramme

Protein analysis realized according to Kjedahl method. Briefly, homogeneous plant samples (1 gr) were placed in digestion tube. Added 7 g catalyst and 20 mL H_2SO_4 with 3 to 5 anti-bumping glass bead. Also prepare a tube was contained the above chemicals except plant sample as blank. The digestion tube was placed in digestor. Placed tube digested at about 200-250 °C for 15 mins before. The digestor was run temperature around 350-380 ºC until solution is light green (30-45 minutes for one plant sample). Removed tube and leaved to stand until sample is cooled after that added about 150-200 ml distilled water. Digested sampels were transferred to distillation apparatus. Added 75 mL 40% NaOH into digested sample (1-2 pieces of zinc (Zn) granules were placed to prevent explosions). 50 mL 2% boric acid into 500 mL flask with 5-6 drop indicator were dispensed. Placed the flask under the condenser, ensured that the condenser tip immersed in the boric acid solution. Distillation process was done until collected approximately 150 ml distillate about 10-20 mins. At the end of distillation process, the blue-violet boric acid solution turned green. The sample was titrated with standard 0.1 N HCl.

For the ash content, sample was weighed about 2 g into the porcelain cruvible, and then pre-burning sample was kept at 550 °C in the ash oven. The amount of ash content was calculated as percantage

2.7. Determination of Fatty Acid Composition

Fatty acid composition was analysed by GC-MS. Before the GC-MS, fatty acids were converted methyl ester form according to TS EN ISO 12966 method (TSE, 2014). Sample was weighed about 100 mg, added above 5 mL n-hexane and 2N KOH with 100 µL methanol. Methyl esters were analysed on a fused silica column (30 mm x 0.25 mm x 0.2 µm) with helium as a carrier gas. The temperature was started 50 °C for 1 min then it was raised at 25 °C/min to 230 °C. Finally, the temperature was held 230 °C for 7 min. The injection volume was 1 µL and split ratio 1:20. The injection temperature was 250 °C. The components were identified by comparison of their mass spectra with charecteristic features obtained with the NITS and Wiley Library spectral data bank.

2.8. Determination of Antimicrobial Activity

To determine the antimicrobial activity of the extracted *P. asplenioides*, a disk diffusion method was used (Matuschek et al., 2014). For antimicrobial activity analysis, different ten (10) bacterial species and different three (3) yeast species were used. All the test microorganisms were obtained from Research and Application Center of the Centreal Research Laboratory of Gümüşhane University. For each assay 10 mL of H_2O was added over about 1 g extract and suspended with stirring.Vigorous agitation was performed to homogenize the suspension. Then, 20 µL of the extract water mixture was soaked on the antimicrobial discs and placed in petri dishes in which bacteria and yeasts were inoculated. For antimicrobial activity, bacterial and yeast cultures grown in Nutrient Broth (Merck, Germany) at 36°C in 24 hours and in Malt Extract Broth (Merck, Germany) at 27°C in 48 hours were used, respectively. After the incubation, the results of antimicrobial activity analysis were determined by measuring the transparent zones formed around the discs.

3. Results

3.1. Antioxidant capacity

There are many methods of measuring the antioxidant capacity in natural product. A single method is usually insufficient when determining antioxidant activity. Therefore, this capacity could be evaluated by a variety of methods pertaining to different mechanisms. In this study, FRAP, DPPH, ABTS and TAC methods were used to investigate antioxidant capacity of *P. asplenioides* . Antioxidant activity of the selected plant was given Table 1. The DPPH radical scavenging activity test is based on the degree of the decolourization of the purple coloured solution. DPPH values of extract was found 2285.49 mg AA/kg. DPPH % inhibition rate of the selected sample was determined as 58.98 %. FRAP values of the sample was found 4729 mg $FeSO_4$/kg in this study. If ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay, which is known as Trolox equivalent antioxidant capacity (TEAC), is high value, it means a greater antioxidant potential of sample. ABTS value of extract in our study was found as 3202 mg Trolox/ kg. ABTS % inhibition was found 24.10 %. Total amount of the antioxidant (TAC) substance was determined 655.59 mg AA/kg. TPC is generally

used as an antioxidant test. According to Table 1 it observed that the TPC value was found 430.67 mg GAE/kg, while TFC value of extract was found 322.33 mg GU/kg. When the results of all antioxidant analysis are examined, it can be sait that *P. asplenioides* extract is moderately potential antioxidant source.

Table 1. Antioxidant activity of the *P. asplenioides*

Sample	TPC	DPPH		FRAP
	mg GAE/kg	**mg AA/kg**	**% inhibition**	**mg $FeSO_4$ /kg**
Plagiochila asplenioides	430.67 ±1.88	285.49 ±8.34	58.98 ±1.68	4729.0 ±4.09
	TAC	**ABTS**		**TFC**
	mg AA/kg	**mg Trolox/ kg**	**% inhibition**	**mg GU/kg**
	655.59 ±3.79	3202.38 ±7.11	24.10 ±0.83	322.33 ±6.28

3.2. Mineral content

Mineral content of the plant is important of food and diet. The mineral composition of *P. asplenioides* extract was examined Table 2. Mineral elements of extract were found in varying proportions. According to result, Calcium (Ca) ion was found to very high (130034.76 mg/kg), Crom (Cr) ion (8.59 mg/kg) was found to very low compared to other elements. Ni and Co ions were detected below limit of quantification (LOQ). Second most abundant ion of the *P. asplenioides* extract was determined potassium (91613.15 mg/kg). It is obvious that *P. asplenioides* extract is high mineral content.

Table 2. Mineral contents of the *P. asplenioides*

Sample	Fe	Ca	Zn	Cd	Mg	Cu	Co
Plagiochila asplenioides	7414.06 ±41.12	130034.76 ±61.73	130.83 ±11.19	<LOQ	520.44 ±17.43	17.19 ±1.33	<LOQ
	Ni	**Al**	**Mn**	**Pb**	**Cr**	**K**	
	<LOQ	2010.77 ±56.87	972.12 ±16.51	349.50 ±81.12	8.59 ±0.66	91613.15 ±267.98	

Note: Results are presented as means minerals mg/kg

3.3. Protein, ash and total lipid contents

Protein, ash and total lipid contents of the selected plant was given Table 3. Protein, ash and total lipid contents were found 10.27 %, 33.83 % and 1.67 %, respectively. According to this results, *P. asplenioides* had a high level of ash, moderate level of protein and lower level of lipid.

Table 3. Total protein and ash content of *P. asplenioides*

Sample	Total Protein (%)	Ash Content (%)	Total Lipid Content (%)
P. asplenioides	10.27 ±0.32	33.83 ±2.12	1.67 ±0.03

3.4. Fatty acid composition

Fatty acid composition of extract from *P. asplenioides* was presented in Table 4 and Figure 1. Result of the analysis was identified 9 fatty acid compounds. Oleic acid was determined to be major fatty acid (47.67 %). Palmitic acid was detected as the second most abudant fatty acid in this sample (38.93 %) (Fig. 1).

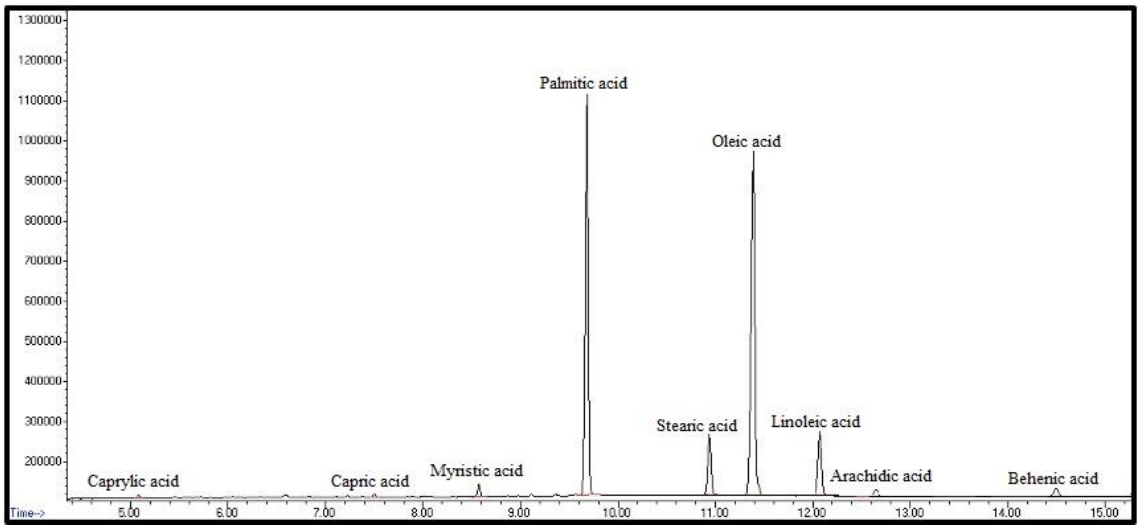

Figure 1. GC-MS chromatograms of the fatty acid composition from *P. asplenioides*

Saturated fatty acids represented 49.59 % of total fatty acids, with the palmitic (38.93 %) and stearic acid (6.68 %) as the main acids. Total monounsaturated acid was determined very near value of saturated fatty acids, but polyunsaturated fatty acid was lower than saturated acids in the *P. asplenioides.*

Table 4. Fatty acid composition of *P. asplenioides*

Fatty acid	**Area (%)**
Caprylic acid (C8:0)	0.52 ±0.02
Capric acid (C10:0)	0.27 ±0.06
Myristic acid (C14:0)	1.05±0.5
Palmitic acid (C16:0)	38.93±2.05
Stearic acid (C18:0)	6.68 ±1.02
Arachidic acid (C20:0)	0.97 ±0.04
Behenic acid (C22:0)	1.17 ±0.07
∑ Saturated fatty acid	**49.59**
Oleic acid (C18:1)	42.67 ±6.99
∑ Monounsaturated fatty acid	**42.67**
Linoleic acid (C18:2)	7.74 ±0.19
∑ Polyunsaturated fatty acid	**7.74**

3.5. Antimicrobial activity of the *P. asplenioides*

Table 5 was presented the results of the antimicrobial activity on the *P. asplenioides* extract. It was assumed that there was a strong effect when the *P. asplenioides* extract showed an inhibition zone of > 20 mm, while this effect was moderate when 12 < inhibition zone < 20 and weak inhibition zone < 12 mm.

Table 5. Antimicrobial activity results of the *Plagiochila asplenioides*

Bacteria sp.	**Sample**	**Penicilin G(10 mg)**
Aeromonas hydrophila ATCC 35654	-	34±0,01
Bacillus cereus ATCC 9634	-	30±0,01
Bacillus subtilis	-	34±0,01
Enterococcus faecalis ATCC 29212	-	32±0,01
Escherichia coli ATCC 25922	4.38±0.01	34±0,01
Escherichia coli O157:H7 35150	-	34±0,01
Listeria monocytogenes ATCC 7644	-	30±0,01
Salmonella typhimurium ATCC 23566	4,12±0,01	34±0,01
Shigella flexneri ATCC 12022	-	30±0,01
Staphylococcus aureus ATCC 25923	-	38±0,01
Yeast-Mould		
Aspergillus flavus ATCC 46283	-	25±0,01
Candida albicans ATCC 10231	-	22±0,01
Saccharomyces cerevisiae S288C	-	14±0,01

Looking at Table 5, it showed activity against only two bacteria (*E. coli*: ATCC 25922 and *S. typhimurium* ATCC 23566) out of total 10 bacteria. Also, it was determined that extract obtained from *P. asplenioides* was not effective against Yeast-Mould. As shown in Table 5, the best antimicrobial activity was obtained against *E. coli.* Therefore, it can be said that plant extract are weak effect against selected of Table 5 microorganism.

4. Discussion and Conclusion

When the literature is examined, it seen that studies about bryophytes are very limited (Sabovljevic et al., 2012). With the regard of the antioxidant, antimicrobial and some chemical compound of *P. asplenioides*, literature data was not found, as well. Therefore, similar analysis of different bryophte species were evaluated in this section.

In the study of Gökbulut et al., (2012), it was determined that methanol and ethyl acetate extracts of *Marchantia polymorpha* L. was exhibit moderate antioxidant activity in the analysis by DPPH (0.4495 mg/mL for methanol and 0.2756 mg/mL for ethyl acetate) and ABTS (0.2441 mg/mL for methanol and 0.2126 mg/mL for ethyl acetate) (Gökbulut et al., 2012). Hanif et al., (2014) examined the evaluation of two bryophytes (*Funaria hygrometrica* Hedw. and *Polytrichum commune* Hedw.) as a source of natural antioxidant. In the study, they reported that the DPPH analysis *Funaria hygrometrica* and *Polytrichum commune* were found 94.7% and 94.4% respectively. Also, the authors reported that the ABTS assay of *Funaria hygrometrica* and *Polytrichum commune* were found 71.06 and 97.5 mM Trolox, respectively (Hanif et al., 2014). In another study about total phenol, antibacterial and antioxidant activity of four bryophyte species (*Plasteurhynchium striatum* (Spruce) M. Fleisch, *Palamocladium euchloron* (Bruch ex Müll. Hal.) Wijk & Margad., *Cratoneuron filicinum* (Hedw.) Spruce and *Campyliadelphus chrysophylus* (Brid.) R.S. Chopra), it determined total phenolic content values ranged from 0.027 to 0.00055 mg GAE/g, while DPPH values ranged from 65.11% to 51.94% (Öztürk et al., 2021). Compared with this study, our antioxidant and TPC value was found generally higher.

The source of major elements are related with natural processes, while presence of many trace elements can be due to environmental pollution. Pd and Cd are associated with directly environmental pollution among the metal. In this study, Pd and Cd were found low concentration, but concentration of major elements and essential trace element were found highest concentration. According to Klavina et al., (2012) it means that pollution that is connected with the trace elements is low in regions were the bryophytes were collected (Klavina et al., 2012).

Among all metabolites in bryophytes, lipids are one of the most important metabolite. These metabolites play important role such as energy storage, membrane formation, cell signaling, functioning and environmental adaptation (Christie et al., 2012). In general, the total lipid content of bryophytes ranges from 1 to 9.1 % of dry weight. These values can change depending on the region and growth condition (Dembitsky, 1993). In the performed study total fat content of *P. asplenioides* is similar of literature. Fatty acid from healthy living bryophytes normally does not accumulate in tissue (Liu et al., 2019). According to literature, palmitic acid (16:0) and stearic acid (18:0) are found most abundant fatty acid in bryophytes, while lauric acid (12:0) and myristic acid (14:0) are also found in a limited amount. In addition, pentadecanoic acid (15:0) and margaric acid (17:0) are found in trace amounts in some bryophytes. In particular, mono- and polyunsaturated fatty acid like oleic acid (18:1), linoleic acid and α-linoleic acid are major compounds among the fatty acid in all bryophytes species (Prins, 1982). The literature's about fatty acids are largely in parallel with the performed this study.

It is known that bryophyte species have antimicrobial activity, also potential antimicrobial activity of bryophytes vary from species to species (Gül et al., 2023). Gül et al. (2023) reported that ethyl acetate and hexane extracts of a bryophyte species, *Leucodon sciuroides* (Hedw.) Schwägr, have no effective antimicrobial activity using the ADD method in their study, but methanol extract from the bryophyte species have determined to have antifungal potential against *C. albicans* species (Gül et al., 2023). On the other hand Ilhan et al. (2006) determined that acetone extracts of *Palustriella commutata* (Hedw) Ochyra have more effective antibacterial activities on Gram-negative bacteria than the Gram-positive strains tested, using the ADD method (Ilhan et al., 2006). Frahm has also reported that aqueous extract of few bryophytes have some inhibitory effect on the growth of *Escherichia coli* as tested on plates (Frahm and Kirchhoff, 2002). Similarly, in performed study, methanol extract obtained from *P. asplenioides* were found to have antimicrobial

effects on *E.coli* and *S.typhimurium* one of the Gram-negative bacteria tested.

In conclusions, phytochemical profile, antioxidant, antimicrobial, fatty acid and mineral profile of *P. asplenioides* were evaluated in this study. Our findings reveal that *P. asplenioides* extract was show moderate antioxidant activity, total phenolic and flavonoid content. In this respect, *P. asplenioides* may think as the potential source of antioxidant. As a result of the GC-MS analysis conducted on *P. asplenioides* extract for fatty acids, our findings demonstrate that the dominant compounds were oleic acid and palmitic acid. Nevertheless mineral analysis results was showed that *P. asplenioides* can accumulate large quantities of certain elements such as Fe, Ca, Al and K. From environmental point of view, this plant may used as pollution indicator. Ash content, total protein and lipid content of sample was determined within the average range. In terms of nutritional, *P. asplenioides* can say not suitable for this. The extract obtained from the *P. asplenioides* showed the weak activity against *E. coli* and *S. typhimurium.* Finally, it was demonstrated the phytochemical, antioxidant and antimicrobial activities and these related data may encourage new studies in the future. Result of this study may valuable contribution in the literature as well because of first study.

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