Chemical Constituents and Bioactivity Studies of Two Polytrichaceae Species, *Polytrichum formosum* Hedw. and *Polytrichum commune* Hedw.

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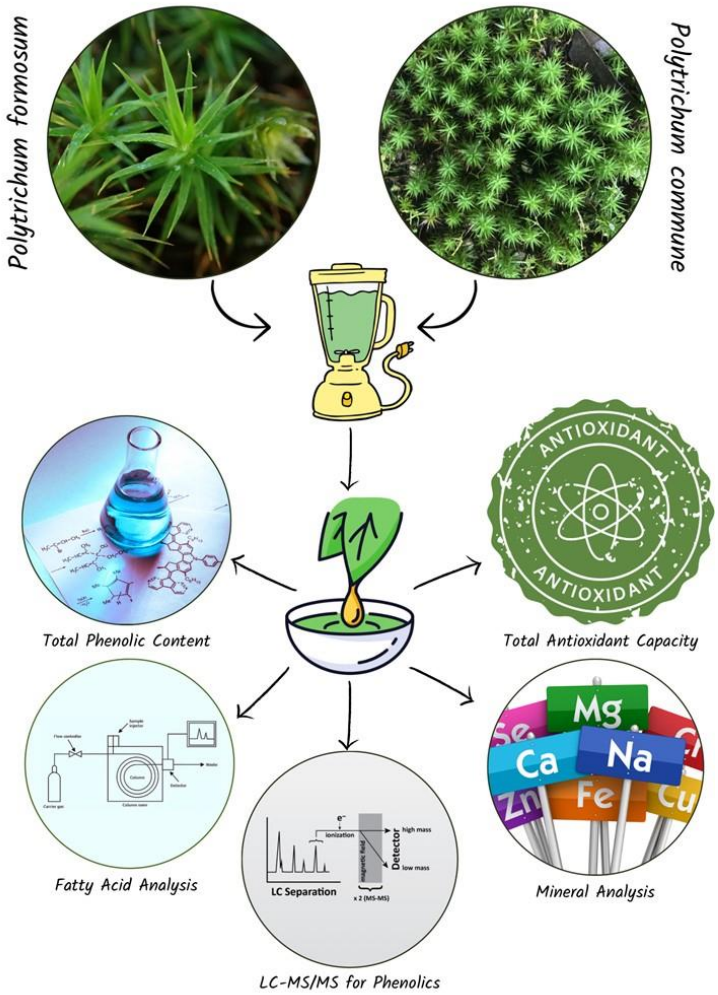
GRAPHICAL ABSTRACT



Chemical Constituents and Bioactivity Studies of Two Polytrichaceae Species, *Polytrichum formosum* Hedw. and *Polytrichum commune* Hedw.

Onur Tolga Okan *

This study aimed to assess the chemical composition, specifically metal content, phenolics, and fatty acids, of two bryophyte species, *Polytrichum formosum* and *Polytrichum commune*, which belong to the Polytrichaceae family. The bioactivities of these species were also evaluated. Microwave plasma atomic emission spectroscopy was used to analyze 13 elements, revealing the presence of 10 elements at varying concentrations in both species. Iron (Fe) was the most prominent element in *P. formosum*, with a concentration of 7610 mg/kg, whereas calcium (Ca) was the most prominent element in *P. commune*, at a concentration of 8430 mg/kg. The high-performance liquid chromatography mass spectrometry analysis revealed the presence of 53 phenolic compounds, with quinic acid, protocatechuic acid, and fisetin being the most prominent with concentrations of 4.45, 2.26, and 1.46 mg analyte/g extract, respectively. When evaluated for total phenolic content, *P. commune* had a higher concentration of phenolic compounds than *P. formosum*. The fatty acid profile obtained *via* gas chromatography-mass spectrometry analysis revealed higher concentrations of oleic acid (36.6% for *P. formosum* and 38.0% for *P. commune*) and palmitic acid (35.5% for *P. formosum* and 33.8% for *P. commune*). Three independent assays assessed the antioxidant capacities of both plants, which revealed significant antioxidant potential in both species.

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Keywords: Antioxidant; Fatty acid; Phenolic profile; Polytrichaceae; Metal analysis

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INTRODUCTION

Recently there has been growing interest in the usage of natural products for various industries, especially in the health sector. Bryophytes growing in forested areas have emerged as important non-wood forest products. According to the theory of evolution, bryophytes are the earliest terrestrial species and are believed to be the first true plants. They hold a direct relationship between algae and vascular plants (Goffinet 2000; Glime 2007). The plant division known as bryophytes comprises three primary classifications: Bryophyta, which includes approximately 14,000 species of mosses; Marchantiophyta, which includes approximately 6,000 species of liverworts; and Anthocerotophyta, which includes approximately 300 species of hornworts. The global diversity of bryophyte species exceeds 20,000 (Glime 2017). Despite the extensive geographical distribution of bryophytes, limited chemical studies have been conducted on this taxonomic group (Klavina *et al*. 2012). Furthermore, chemical analyses have only been conducted on 2% of

mosses and 6% of liverworts within the bryophyte group (Klavina *et al.* 2018). Some researchers have argued that the limited research on bryophytes is due to their unsuitability for human consumption (Asakawa *et al.* 2013; Commisso *et al*. 2021). However, it has been reported that bryophytes are extensively utilized in mainland China for the treatment of various illnesses, including burns, bruises, external wounds, snake bites, pulmonary tuberculosis, neurasthenia, fractures, uropathy, and pneumonia. Consequently, researchers worldwide have begun to investigate secondary metabolites of bryophyte species. Bryophytes are rich in valuable secondary metabolites, such as terpenoids, flavonoids, and bibenzils, which are directly corelated with antioxidant capacity of them (Okan 2023).

The bioactive properties and chemical composition of *Polytrichum* species are relatively understudied. The literature review presented herein encompasses the scientific findings from the past seven decades. Pharmacological investigations have demonstrated that ethanol and acid extracts of *Polytrichum juniperum* Hedw can effectively inhibit the growth of implanted sarcoma cell line 37 in CAF1 mice (Belkin *et al.* 1952; Cianciullo *et al*. 2022). Additionally, Ohioensin A-E, a novel benzonaphthoxanthenone isolated from *P. ohioense*, was found to be toxic to various cancer cells (Zheng *et al*. 1993). Three novel benzonaphthoxanthenones and two novel cinnamoyl bibenzyls were isolated through bioassay-directed fractionation of an ethanol extract of *P. pallidisetum*, which significantly inhibited the growth of the melanoma cell line RPMI-7951, glioblastoma cell line U-251, and multiple myeloma cell line MM-1 (Zheng *et al*. 1994). *Polytrichum commune* L. Hedw (PCLH) has been used in traditional Chinese medicine to treat a range of conditions including fever, hemostatic and traumatic injuries, pneumonia, uterine prolapse, and lymphocytic leukemia (Zhao *et al.* 2018). Two new compounds, communins A, B, and H, isolated from PCLH, have shown potential as anticancer agents by effectively damaging a small panel of cancer cell lines, such as the lung cancer cell line A-549 and leukemia cell line 6T-CEM (Fu *et al*. 2009). The *P. commune* species has been used as a folk remedy to treat bleeding, pneumonia, fever, hemostatic and traumatic injury, uterine prolapse, and various types of cancer, including glioma (Cheng *et al*. 2012, 2013). Previous studies have shown that *P. commune* has anti-leukemic properties that are thought to be related to its ability to regulate intracellular Ca^{2+} homeostasis and induce mitochondria-dependent apoptosis (Cheng *et al.* 2012, 2013). Hanif *et al*. (2014) reported the antioxidant properties of *P. commune*. In addition, it has been traditionally used in the Tujia minority region of China to treat memory decline and other related diseases. Recently, Guo *et al*. (2020) isolated two new and rare benzonaphthoxanthenones from *P. commune* and found that they exhibited *in vitro* anti-neuroinflammatory activity.

Some studies have also been reported on these species in non-English languages; however, limited information is available on the genus *Polytrichum*. Therefore, in the present study, these two species were chosen to investigate their bioactivities and chemical composition to obtain comparative data on both species and to identify additional sources of biomolecules that can be utilized to explore further opportunities to harness the full potential of these valuable species.

The primary objective of this study was to investigate the antioxidant, antimicrobial, and nutritional characteristics, as well as the chemical compositions of the bryophyte species of *Polytrichum formosum* Hedw., and *Polytrichum commune* Hedw. Based on literature review, no prior studies have evaluated the chemical composition, fatty acids, antioxidants, and antimicrobial properties of these two species.

EXPERIMENTAL

Chemical and Reagents

The following chemical substances were obtained from Sigma (St. Louis, MO, USA): Folin-Ciocalteu's phenol reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, monobasic sodium phosphate, ammonium heptamolybdate tetrahydrate, gallic acid, DPPH, potassium persulfate ($K_2S_2O_8$), Trolox(±) (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), sodium nitrite ($NaNO_2$), and aluminum chloride ($AlCl_3$). Standard fatty acid compounds were obtained from Merck (Darmstadt, Germany), and standard phenolic compounds were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol (HPLC grade), sulfuric acid, sodium acetate trihydrate, glacial acetic acid, hydrochloric acid (HCl), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$), and sodium hydroxide (NaOH) were purchased from Merck. The remaining chemicals used in this study were obtained from Merck (Darmstadt, Germany) *via* authorized local distributors.

Plant Materials

The specimens of *Polytrichum formosum* Hedw. and *Polytrichum commune* Hedw. used in this study were collected from Kayabaşı Plateau, Düzköy, Trabzon, (40°50'42.56 "N, 39°28'09.57 "E, A: 1756 m) on 22.09.2023, in *Picea orientalis* (L.) Link. (spruce) forest near a stream in wet soil. The specimens were cleaned and stored in the Herbarium under the Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon, Türkiye. The collected plants were carefully cleaned and washed with distilled water to eliminate soil or other impurities, and the samples were dried at room temperature in a shaded area.

Extraction Process of the Samples

Approximately 2.5 g of the material was combined with 50 mL 99% methanol and blended for 5 min in a blender. The mixture was then placed in a Falcon tube and stirred continuously at room temperature for 24 h using a shaker (Heidolph Promax 2020; Schwabach, Germany). Following filtration through filter paper (Whatman no. 1), the particles were concentrated using a rotary evaporator (IKA-Werke, Staufen, Germany) at a temperature of 40 °C. The resulting residue was dissolved in methanol to a predetermined final concentration and subsequently stored at a temperature of 4 °C.

Antioxidant Activity Analysis

Total antioxidant capacity (TAC)

Total antioxidant capacity of *P. formosum* Hedw. and *P. commune* Hedw. extracts were determined using the phosphomolybdenum assay, as previously described (Prieto *et al*. 1999). The method involved adding 2500 µL of deionized water to 500 µL of extract and mixing it with 1000 µL of phosphomolybdenum reagent (containing 28 mM monobasic sodium phosphate and 4 mM ammonium heptamolybdate tetrahydrate in 0.6 M sulfuric acid) in capped test tubes. The mixture was left to incubate for 90 min in a water bath at 95 °C, after which the absorbance at 695 nm was measured at room temperature, with ascorbic acid serving as the reference substance.

Free radical scavenging activity (DPPH)

The DPPH scavenging activity was evaluated using the method developed by Okan *et al*. (2018). Specifically, 100 µL of the extract solution was combined with 3000 µL of a freshly prepared 10 mM DPPH solution in methanol. The mixture was left undisturbed in the dark at room temperature for 30 min, allowing any reaction to occur. The absorbance was measured at 517 nm using a spectrophotometer, and the values were expressed as ascorbic acid (mg/kg).

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was performed following the method outlined by Ou *et al.* (2002). To conduct the FRAP activity assay on plant extracts, a fresh FRAP reagent was created by mixing 300 mM sodium acetate buffer solution (pH: 3.6), 10 mM aqueous TPTZ solution in 40 mM HCl, and 20 mM aqueous $FeCl_3$ solution in a ratio of 10:1:1. Specifically, 250 µL of the extract solution was combined with 2750 µL of the newly prepared FRAP reagent. The mixture was incubated at 37 °C for 15 min before being analyzed using a spectrophotometer. The absorbance was measured at 593 nm relative to that of the control, and the results are expressed as mg $FeSO_4$/100 g.

$ABTS^{+\bullet}$ assay protocol

The $ABTS^{+\bullet}$ radical cation stock solution was prepared by mixing 7 mM aqueous $ABTS^{+\bullet}$ and 2.45 mM potassium persulfate in equal volumes and allowing the mixture to sit in the dark for 120 min at room temperature. The resulting solution was diluted with methanol to achieve an absorbance of 0.700 ± 0.020 at 734 nm and room temperature, resulting in an $ABTS^{+\bullet}$ working solution. A 150 µL sample of the extract was mixed with 2850 µL of the ABTS solution and then transferred to a spectrophotometer cuvette for measurement at 734 nm. The results were compared with those of the control and expressed as mg Trolox/kg using the Trolox standard curve (Pellegrini *et al.* 2003).

Total phenolic content (TPC)

Folin-Ciocalteu reagent was used to determine the total amount of phenolic compounds in the plant extracts (Kasangana *et al.* 2015). Specifically, 300 µL of the dry extract was combined with methanol and then added to 200 µL of 2N Folin-Ciocalteu reagent and 600 µL of 10% Na_2CO_3. The mixture was left to sit in the dark for 120 min at room temperature, and the absorbance of the mixture was measured at 760 nm using a spectrophotometer (Unicam UV2-100; ATI Unicam, Canada). A calibration curve was prepared using gallic acid, and the results are expressed as gallic acid equivalents (mg GAE/g dry extract).

Total flavonoid content (TFC)

The total flavonoid content in the sample was evaluated using the method developed by Fukumoto and Mazza (2000) with certain modifications. Initially, 500 µL of the extract was dissolved in 3200 µL methanol, constituting 30% of the volume. Subsequently, 150 µL of 0.5 M $NaNO_2$ and 150 µL of 0.3 M $AlCl_3$ were added to a test tube. After a period of 5 min, 1.0 mL of 1 M NaOH was added to the test tube, and the mixture was incubated at room temperature for 10 min. Finally, the absorbance was measured against a blank at a wavelength of 506 nm and the results were expressed as the amount of quercetin equivalents (QE) per kilogram of weight measured in milligrams (mg QE/kg).

Determination of Total Lipid, Protein, and Ash Contents

The method of Yang *et al.* (2020) was used with certain modifications for the determination of total lipid content. A sample of 5 g of material was placed in a cellulose extraction cartridge and immersed in a cotton wool plug. This cartridge was then inserted into the Soxhlet chamber, where it was placed in a thermostatic water bath set at 60 °C, which was connected to a tared distillation flask containing 100 mL of n-hexane and 2-3 boiling glass stones. The extraction process was continued for 16 h, after which most of the solvent was removed using a rotary evaporator. The remaining residue was transferred to an oven at 103 °C and maintained until it reached constant weight. The crude oil content was calculated as a percentage.

The analysis of protein was conducted through the application of the Kjeldahl method (Okan 2023). A total of 1 g of homogenous plant sample was introduced into a digestion tube, followed by the addition of 7 g of catalyst and 20 mL of H_2SO_4 comprising 3-5 anti-bumping glass beads. A blank tube containing the identical chemicals, but without the plant sample, was prepared as well. The digestion tube was placed in a digestor and heated at 200 to 250 °C for 15 min. The digestor was then heated at a temperature of 350 to 380 °C until the solution turned light green, which took 30 to 45 min for one plant sample. After cooling, about 150 to 200 mL of distilled water were added. The digested samples were then transferred to a distillation apparatus, where 75 mL of 40% NaOH was added together with 1 to 2 pieces of zinc granules to prevent an explosion. Fifty milliliters of 2% boric acid were dispensed into a 500-mL flask with 5 to 6 drops of indicator, and the flask was placed under a condenser with the tip immersed in the boric acid solution. The distillation process was carried out until approximately 150 mL of distillate was collected over 10 to 20 min. At the end of the distillation process, the blue-violet boric acid solution turned green. Lastly, the sample was titrated with a standard 0.1 N HCl solution.

To determine the ash content, 2 g of sample was weighed and placed in a porcelain crucible. The pre-burned sample was then heated to 550 °C in an ash oven. The ash content was calculated based on these measurements.

Mineral Analysis

The mineral contents of both *P. formosum* Hedw. and *P. commune* Hedw. were analyzed using microwave plasma atomic emission spectroscopy (MP-AES) method, which involved digesting the plant samples in a closed microwave digestion system (Milestone Start D model; Milestone, Sorisole, Italy) with appropriate solvent mixtures (Baltacı *et al.* 2024). To begin the process, 0.5 g of plant material was added to Teflon vessels along with 6 mL of HNO_3 and 2 mL of H_2O_2. The temperature was then increased gradually from 50 to 200 °C, and the mixture was allowed to cool before being transferred to a 100-mL volumetric flask. The volume was adjusted to 50 mL with distilled water, and µg/mL solutions were prepared from 1000 ppm solutions of Fe, Ca, Zn, Cd, Mg, Cu, Co, Ni, Al, Mn, Pb, Cr, and K metal ions. The sample was then filtered through a 0.45-micron cellulose filter (Whatman) before mineral determination using the NMKL 170 and NMKL 161 methods.

Analysis of Fatty Acids Methyl Esters

The protocol used was modified from TS EN ISO 12966-4 (2015). In accordance with this protocol, 100 mg of samples from all solids, oils, and products derived from fatty materials were weighed using a precision balance and placed in 20-mL screw-capped glass tubes. Next, 2 N KOH and 5 mL of n-hexane, along with 100 µL methanol, were added to

the tubes, which were then vortexed for 1.0 min and centrifuged for 5 min. The clear upper portion was transferred to 1.8-mL vials and placed in the gas chromatograph (GC) device for analysis, in accordance with TS EN ISO 12966-4 (2015). The chromatographic conditions of the GC-mass spectrometry (MS) instrument (Agilent-7980; Agilent, Santa Clara, CA, USA) with flame ionization detector (FID) were as follows: set temperature of 250 °C, pressure of 21.231 psi, total flow of 42.398 mL/dk, injection volume of 1 µL, and a split rate of 1:20. A Restek Rtx-2330 column (fused silica) that measured 30 mm × 0.25 mm × 0.2 µm (Cat. No. 10723, Serial No. 1081624) was used to separate fatty acids. The column furnace program was set as follows: The initial temperature was 50 °C, which was maintained for 1.0 min, and then increased to 200 °C at a rate of 25 °C/min and maintained at this temperature for 1.0 min. The temperature was then increased to 230 °C at a rate of 3 °C/min and maintained for 27 min with a total run time of 45 min. The detector conditions were set at an FID temperature of 280 °C, H_2 flow rate of 40 mL/min, air flow rate of 450 mL/min, helium flow rate of 25 mL/min, MS Detector MS Quad set at 150 °C, MS Source at 230 °C, Aux-2 Temperature of 230 °C, and solvent delay of 3.75 min.

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis for Phenolics

A Shimadzu-Nexera UHPLC equipped with a tandem mass spectrometer was employed to quantitatively assess 53 phytochemicals. The ultra-high performance liquid chromatography system was configured with an auto sampler (SIL-30AC model), column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and degasser (DGU-20A3R model) that utilized reversed-phase technology. The conditions for chromatography were fine-tuned to attain optimal separation of 53 phytochemicals and to overcome suppression effects. Various columns, including Agilent Poroshell 120 EC-C18 model (150 mm × 2.1 mm, 2.7 µm) and RP-C18 Inertsil ODS-4 (100 mm × 2.1 mm, 2 µm), as well as different mobile phases (B), such as acetonitrile and methanol, were tested and applied until optimal conditions were established. Additionally, various mobile phase additives, including ammonium formate, formic acid, ammonium acetate, and acetic acid, and different column temperatures, such as 25, 30, 35, and 40 °C, were tested to achieve the desired results. Chromatographic separation was performed using an Agilent Poroshell 120 EC-C18 analytical column, which measured 150 mm in length and 2.1 mm in diameter with a particle size of 2.7 µm. The temperature of the column was set at 40 °C. The elution gradient was composed of eluents A and B, where A was a mixture of water, 5 mM ammonium formate, and 0.1% formic acid, and B was a mixture of methanol, 5 mM ammonium formate, and 0.1% formic acid. The gradient elution profiles used were 20% to 100% B from 0 to 25 min, 100% B from 25 to 35 min, and 20% B from 35 to 45 min. The solvent flow rate was set at 0.5 mL/min, and the injection volume was 5 µL.

A Shimadzu LCMS-8040 spectrometer equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes was used to perform mass spectrometric detection. The Lab Solutions software (Shimadzu) was used to acquire and process the liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) data. The multiple reaction monitoring (MRM) mode was employed for the quantification of phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on screening of specified precursor phytochemical-to-fragment ion transitions. The collision energies (CE) were optimized to generate optimal photochemical fragmentation and maximal transmission of the desired product ions. The MS operating conditions included a drying gas (N_2) flow of 15 L/min, a

nebulizing gas (N_2) flow of 3 L/min, a desolvation line (DL) temperature of 250 °C, a heat block temperature of 400 °C, and an interface temperature of 350 °C.

RESULTS AND DISCUSSION

Determination of Total Polyphenol, Total Flavonoid, and Antioxidant Analyses

The data for the comparative analysis of the total phenolic content (TPC), total flavonoids content (TFC), and total antioxidant content (TAC) in *P. formosum* and *P. commune* are shown in Fig. 1. According to Fig. 1, TPC, TFC, and TAC values for *P. formosum* were 507.33 ± 27.56, 449.01 ± 14.45, and 595.11 ± 48.12 mg GAE/kg respectively.

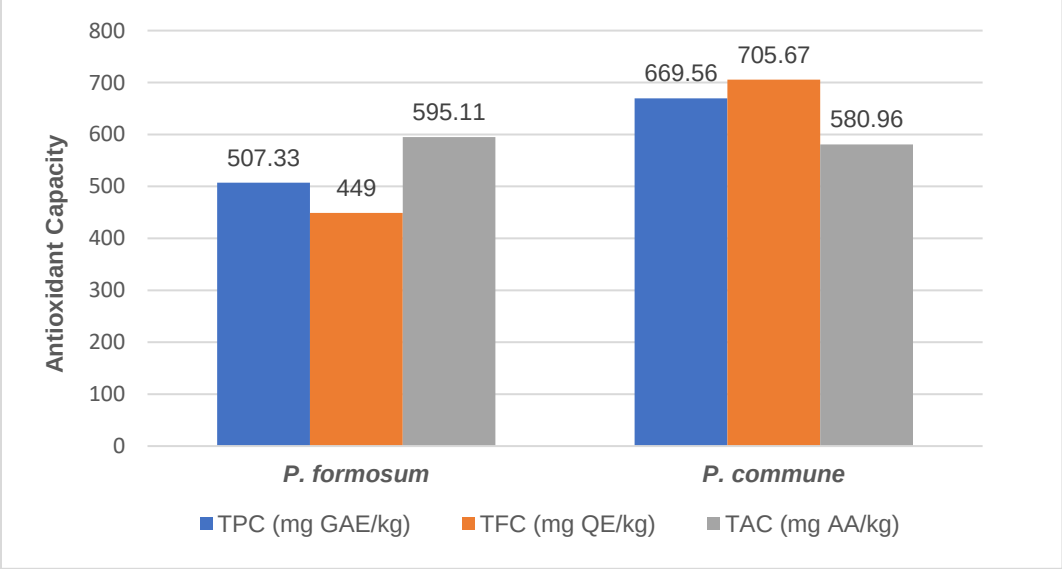

Fig. 1. Total phenolic, flavonoid, and antioxidant contents in *P. formosum* and *P. commune*

Similarly, for *P. commune*, these values were 669.56 ± 66.21, 705.67 ± 89.61, and 580.96 ± 34.59 mg GAE/kg, respectively. The presence of substantial quantities of polyphenols is commonly associated with high antioxidant activity. Given this relationship, it is essential to utilize multiple methods to accurately assess the antioxidant activity of natural products. In this context, relying on a single approach would be insufficient. The results of the antioxidant activities of *P. formosum* and *P. commune* extracts analyzed using the three different methods are presented in Table 1.

Table 1. Antioxidant Activity Determined using ABTS, FRAP, and DPPH Assays

Plant	ABTS		FRAP	DPPH	
	% Inhibition	mg Trolox/kg	mg $FeSO_4$/100g	% Inhibition	mg AA/kg
P. formosum	26.91 ± 5.43	3646.83 ± 61.33	681.71 ± 31.05	50.19 ± 11.96	241.81 ± 17.13
P. commune	34.89 ± 6.61	4908.73 ± 84.19	820.92 ± 47.84	70.83 ± 23.77	344.38 ± 19.10

The antioxidant activities of *P. formosum* and *P. commune* were assessed using the ABTS, FRAP, and DPPH assays. The results showed that the ABTS value for *P. formosum* was 3646.83 ± 61.33 mg Trolox/kg, the FRAP value was 681.71 ± 31.05 mg $FeSO_4$/100g, and the DPPH value was 241.81 ± 17.13 mg AA/kg. For *P. commune*, the corresponding values were 4908.73 ± 84.19 mg Trolox/kg, 820.92 ± 47.84 mg $FeSO_4$/100g, and 344.38 ± 19.10 mg AA/kg. Although both selected samples showed prominent antioxidant activity, *P. commune* was found to have stronger antioxidant activity than *P. formosum*. After reviewing the literature, it became apparent that the number of studies conducted on bryophytes is limited (Okan 2023). Furthermore, no studies have reported the antioxidant and chemical compounds in *P. formosum* and *P. commune*. Consequently, a similar analysis of different bryophyte species was conducted, as mentioned in the discussion section. According to Klavina *et al.* (2015) study on the chemical components of *P. commune*, the TPC value was reported to be 800.7 ± 39.1 mg GAE/100 g (Klavina *et al.* 2015). In another study on the chemical components of *P. formosum*, the TPC value was found to be 4.387 ± 0.806% (Chobot *et al.* 2008). In a study conducted to evaluate the antioxidant potential of five different species of moss found in Europe, the DPPH, and ABTS values were determined as follows: 87.69 and 171.50 mol Trolox equivalent (TE)/l for *Brachythecium rutabulum*, 79.66 and 88.59 molTE/l for *Callicladium haldanianum*, 15.02 and 79.00 molTE/l for *Hypnum cupressiforme*, 28.99 and 44.51 molTE/l for *Orthodicranum montanum*, and 117.79 and 215.58 molTE/l for *Polytrichastrum formosum* (Smolińska-Kondla *et al.* 2022).

Determination of Total Lipid, Protein, and Ash Contents

It is widely believed that the scarcity of studies on bryophytes can be attributed to their limited significance as a primary food source (Askawa *et al.* 2013; Commisso *et al.* 2021). The amounts of lipids and proteins in a plant can provide an initial indication of its suitability as a food source. The protein, ash, and total lipid content of the selected plants are shown in Table 2.

Table 2. Total Lipid, Ash Content, and Protein Content

	Total Lipid (%)	Protein content (%)	Ash content (%)
P. formosum	0.80 ± 0.03	14.13 ± 0.14	17.84 ± 0.18
P. commune	4.91 ± 0.09	11.64 ± 0.42	15.29 ± 0.55

The ash contents of medicinal plants are evidence of elements remaining after heating at very high temperatures to remove moisture, volatiles, and organics. Common elements included calcium, magnesium, sodium, and potassium, along with traces of manganese, zinc, and iron. The ash contents of *P. formosum* and *P. commune* were in accordance with the mineral analysis (Table 3), although the values were relatively higher than those reported previously (Das *et al.* 2022). According to research on *P. asplenioides*, the levels of total protein, ash, and total lipids are 10.27%, 33.83%, and 1.67%, respectively (Okan 2023). Bryophytes have been found to have nutritional properties that can have a positive impact on diet, as evidenced by their high protein content, which ranges from 9% to 25% in certain cereals (Çetiner and Bilek 2018), and their fat content, which ranges from 1.9% to 7% (Rosentrater and Evers 2018).

Determination of Mineral Content

The analysis of minerals was conducted on both plant species, and it was found that the same number, 10, of these minerals were present in both species. However, the quantities of these minerals differed (Table 3). The analytical method used for the mineral analysis did not detect any cadmium, cobalt, or nickel at the limit of quantification, which was 0.01 mg/kg. The concentrations of ferrous ions, copper, and aluminum were significantly higher in *P. formosum* than in *P. commune*, while zinc was present in greater quantities in *P. commune* than in *P. formosum*. Calcium levels in *P. commune* were significantly higher than those in P. *formosum*, amounting to more than four-fold the level in the latter. Similarly, lead concentrations in *P. commune* were also observed to be considerably higher than those *in P. formosum*. Chromium and potassium were also considerably higher in *P. formosum* than *in P. commune*, whereas magnesium and manganese concentrations were almost the same in both plants. Based on the authors' thorough review of the available literature, no reports on the minerals present in *Polytrichum* species were found.

Table 3. Mineral Content of the *P. formosum* and *P. commune*

Metal mg/kg	*P. formosum*	*P. commune*
Fe	7613.70 ± 98.21	3171.72 ± 81.19
Ca	2093.24 ± 33.61	8425.91 ± 85.96
Zn	27.59 ± 3.36	64.96 ± 7.99
Cd	< LOQ	< LOQ
Mg	451.95 ± 13.77	432.12 ± 11.15
Cu	5.71 ± 1.19	2.82 ± 1.61
Co	< LOQ	< LOQ
Ni	< LOQ	< LOQ
Al	2989.23 ± 41.28	1275.97 ± 21.12
Mn	129.40 ± 10.04	169.46 ± 13.28
Pb	14.27 ± 5.12	225.00 ± 12.14
Cr	2.85 ± 0.81	1.88 ± 0.19
K	5166.26 ± 109.97	4538.56 ± 95.65
Total Mineral (mg/kg)	64975.95 ± 143.33	58926.41 ± 120.54
Total Mineral (%)	6.50 ± 1.19	5.89 ± 2.21

Therefore, it is not possible to establish a direct correlation between the authors' findings and those of other studies on the same species. However, if these figures are compared with the previous report (Bahadori *et al.* 2019), the mineral concentration of *P. formosum* than *P. commune* is considerably higher. However, some elements, such as Cd, Co, and Ni, were below the LOQ in the present study. Although most of the metals (Fe, Ca, Zn, Al, Mn, and K) were relatively very high with similar ratios, few metals were found in comparable concentrations, that is, Mg, Cu, and Pb. Only Cr was found to be lower than that in the previous data. Because plants are mainly explored to produce solutions for human health, it is pertinent to discuss the source of metals and their importance. The adequacy of Fe prevents anemia, whereas Cu is necessary to shelter the brain and heart from different diseases and anemia attacks. Zinc is necessary for healthy skin and hair, whereas Ca is necessary for bone to refrain from deterioration. Cr reduces glucose tolerance (Gupta 2018). K is important for maintaining normal levels of fluid inside cells,

while the role of Mg is to regulate muscle and nerve function, blood sugar levels, blood pressure, and the production of proteins, bones, and DNA.

Fatty Acid Composition

Fatty acids are commonly found in membrane phospholipids and glycolipids and are incorporated into triacylglycerides (TAGs). Healthy bryophyte tissues generally do not contain free fatty acids (Lu *et al.* 2019).

The fatty acid compositions of *P. formosum* and *P. commune* are presented in Table 4 and Fig. 2. The data presented in Table 4 indicate that both palmitic and oleic acids were found in significant amounts in all the samples. Specifically, in *P. formosum*, the levels of palmitic acid and oleic acid were 35.5% and 36.6%, respectively, whereas in *P. commune*, they were 37.9% and 38.0%, respectively. Despite these differences, both samples contained significant levels of stearic, linoleic, arachidic, and behenic acids.

Table 4. Fatty Acids Analyzed on GCMS (% Area)

Fatty Acid	*IUPAC*	*P. formosum*	*P. commune*
Caprylic acid (C8:0)	Octanoic acid	0.252 ± 0.002	0.368 ± 0.004
Capric acid (C10:0)	Decanoic acid	N.D.	0.446 ± 0.002
Myristic acid (C14:0)	Tetradecanoic acid	1.157 ± 0.03	2.536 ± 0.04
Palmitic acid (C16:0)	Hexadecanoic acid	35.515 ± 3.61	37.855 ± 1.99
Stearic acid (C18:0)	Octadecanoic acid	5.637 ± 0.91	6.047 ± 0.88
Arachidic acid (C20:0)	Eicosanoic acid	5.061 ± 0.13	4.017 ± 0.15
Behenic acid (C22:0)	Docosanoic acid	3.756 ± 0.02	2.952 ± 0.08
Σ Saturated fatty acid		**51.378**	**54.221**
Oleic acid (C18:1)	(Z)-9-Octadecenoic acid	36.628 ± 4.33	38.022 ± 2.20
Σ Monosaturated fatty acid		**36.628**	**38.022**
Linoleic acid (C18:2)	(Z),(Z)-9,12-Octadecadienoic acid	9.995 ± 4.01	7.758 ± 2.07
Σ Polyunsaturated fatty acid		**9.995**	**7.758**

However, the level of caprylic acid was very low in both sample types. Although it was not detected in *P. formosum*, capric acid was exclusively detected in *P. commune* (Fig. 2).

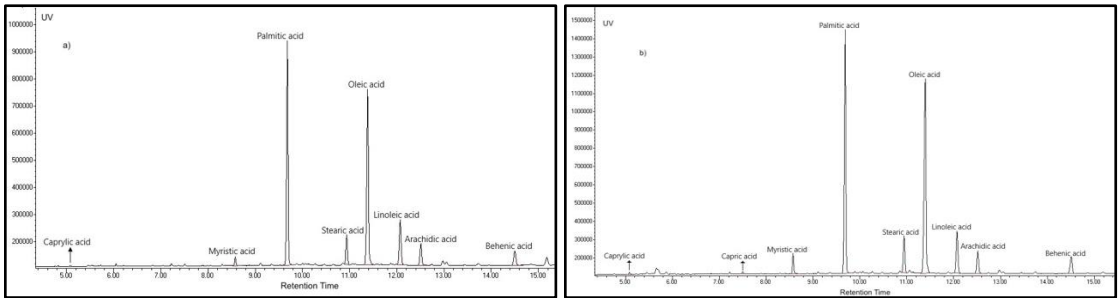

Fig. 2. Chromatograms of the fatty acid compositions from *P. formosum* (a) and *P. commune* (b)

A literature review (Lu *et al*. 2019) showed that bryophytes, like other plant and organism species, are capable of producing common saturated fatty acids such as palmitic acid (16:0) and stearic acid (18:0). Additionally, they contain medium-chain fatty acids,

including lauric acid (12:0), and myristic acid (14:0), although in limited amounts. Furthermore, certain bryophytes have been reported to contain trace amounts of odd-chain saturated fatty acids, such as pentadecanoic acid (15:0) and margaric acid (17:0), which are typically uncommon in nature (Lu *et al*. 2019). The results of the present study were largely consistent with the cited review. A study conducted on extracts obtained from *P. commune* has reported the identification of linoleic acid, behenic acid, stearic acid, myristic acid, arachidic acid, palmitic acid, and oleic acid through chemical analyses, with their respective concentrations being 31.2, 28.7, 25.5, 11.1, 7.9, and 2.66 mg/100 g, in order of the highest to lowest concentration (Klavina *et al.* 2015). In a study conducted on extracted samples from *Eurhynchium praelongum*, *Rhynchostegium murale*, *Brachythecium erythrorrhizon*, and *Amblystegium riparium*, a total of 19 fatty acid components were identified. Palmitic acid was reported to be the major component in *E. praelongum* and *R. murale*, accounting for 25.8% and 20.0%, respectively. Additionally, linolenic acid was a significant component in both species, comprising 20.14% and 19.14%, respectively (Ismaeel *et al.* 2022). However, it has been reported that environmental conditions are the main reason bryophyte species of the same type growing in different locations contain varying amounts and/or different fatty acid components (Chodok *et al.* 2010; Takemura *et al.* 2011). Lu *et al.* (2019) have indicated that environmental factors play a crucial role in the production of polyunsaturated fatty acids in bryophytes. They specifically reported that variables, such as temperature, light, pH, and nutrition, have a significant impact on fatty acids level (Lu *et al*. 2019). Additionally, it is known that the polarity of solvents also affects the ratio and detection of fatty acid components (Ismaeel *et al.* 2022). Therefore, while the components and quantities of fatty acids identified in this study may be consistent with the literature for similar species, differences from the literature findings may arise because of the abovementioned reasons.

Phenolic Content

Phenolic compounds are a broad class of substances widely distributed in plants. These compounds can inhibit the activity of free radicals, which are byproducts of cellular metabolism within the human body and prevent the deformation of DNA (Okan *et al.* 2018). Fifty-six phenolic standards were analyzed using LC-MS (Fig. 3).

The results of this analysis are presented in Table 5 and Fig. 4. In *P. formosum*, quinic acid had the highest concentration (4.45 mg/g) among all detected phenolics, followed by protocatechuic acid (2.26 mg/g). Conversely, in *P. commune*, protocatechuic acid was highest (4.22 mg/g), followed by quinic acid (3.78 mg/g). 4-Hydroxy benzoic acid (1.88 mg/g), *p*-coumaric acid (0.69 mg/g), and fisetin (1.46 mg/g) in *P. formosum* and fumaric aid (0.74 mg/g), protocatechuic aldehyde (1.35 mg/g), 4-OH-benzoic acid (1.16 mg/g), vanillic acid (0.96 mg/g), caffeic acid (0.66 mg/g), vanillin (1.13 mg/g), *p*-coumaric acid (0.86 mg/g), and rutin (1.46 mg/g) were found in considerable quantities. All other phenolics in the list were either not detected or found in minor quantities. In overall comparison, it is observed that quantities of detected phenolics were higher in *P. commune* as compared to *P. formosum*, which is in agreement with antioxidant activity as well.

Table 5. Phenolic Content (LC-MS Method) (mg analyte/g extract)

Standard No	Analyte	*P. formosum*	*P. commune*
1	Quinic acid	4.454 ± 0.23	3.776 ± 0.11
2	Fumaric acid	N.D.	0.745 ± 0.04
4	Gallic acid	0.542 ± 0.03	0.271 ± 0.06
6	Protocatechuic acid	2.259 ± 0.05	4.218 ± 0.29
9	Chlorogenic acid	N.D.	0.639 ± 0.09
10	Protocatechuic aldehyde	0.651 ± 0.01	1.346 ± 0.21
11	Tannic acid	0.018 ± 0.00	0.17± 0.03
12	Epigallocatechin gallate	N.D.	0.396 ± 0.01
14	4-OH-Benzoic acid	1.884 ± 0.14	1.162 ± 0.74
16	Vanilic acid	N.D.	0.965 ± 0.02
17	Caffeic acid	0.431 ± 0.05	0.658 ± 0.08
19	Vanillin	0.467 ± 0.06	1.134 ± 0.51
22	Epicatechin gallate	N.D.	0.237 ± 0.11
24	p-Coumaric acid	0.687 ± 0.02	0.859 ± 0.02
28	Coumarin	0.102 ± 0.10	0.078 ± 0.09
29	Salicylic acid	0.035 ± 0.01	0.061 ± 0.01
33	Rutin	N.D.	1.463 ± 1.03
34	Isoquercitrin	N.D.	0.245 ± 0.09
35	Hesperidin	N.D.	0.448 ± 0.04
40	Cosmosiin	N.D.	0.137 ± 0.07
42	Astragalin	N.D.	0.237 ± 0.01
43	Nicotiflorin	N.D.	0.232 ± 0.06
44	Fisetin	1.461 ± 0.01	3.331 ± 1.07
48	Naringenin	0.015 ± 0.01	0.027 ± 0.01
50	Luteolin	0.01 ± 0.00	0.042 ± 0.01
53	Apigenin	0.018 ± 0.01	0.025 ± 0.01
56	Acacetin	0.082 ± 0.01	0.059 ± 0.05

N.D: Not Detected

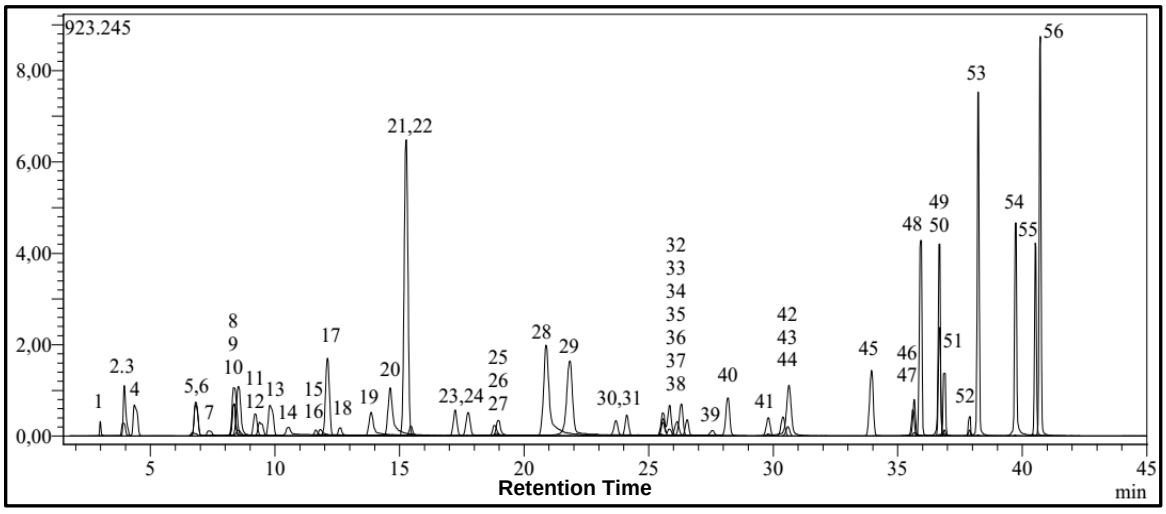

Fig. 3. LC-MS (TIC) chromatogram of 53 standards (1: Quinic acid, 2: Fumaric acid, 3: Aconitic acid, 4: Gallic acid, 5: Epigallocatechin, 6: Protocatechuic acid, 7: Catechin, 8: Gentisic acid, 9: Chlorogenic acid, 10: Protocatechuic aldehyde, 11: Tannic acid, 12: Epigallocatechin gallate, 13: 1,5-Dicaffeoylquinic acid, 14: 4-OH-Benzoic acid, 15: Epicatechin, 16: Vanilic acid, 17: Caffeic

acid, 18: Syringic acid, 19: Vanillin, 20: Syringic aldehyde, 21: Daidzin, 22: Epicatechin gallate, 23: Piceid, 24: *p*-Coumaric acid, 25: Ferulic acid D3 (Internal Standard), 26: Ferulic acid, 27: Sinapic acid, 28: Coumarin, 29: Salicylic acid, 30: Cynaroside, 31: Miquelianin, 32: Rutin D3 (Internal Standard), 33: Rutin, 34: isoquercitrin, 35: Hesperidin, 36: *o*-Coumaric acid, 37: Genistin, 38: Rosmarinic acid, 39: Ellagic acid, 40: Cosmosiin, 41: Quercitrin, 42: Astragalin, 43: Nicotiflorin, 44: Fisetin, 45: Daidzein, 46: Quercetin D3 (Internal Standard), 47: Quercetin, 48: Naringenin, 49: Hesperetin, 50: Luteolin, 51: Genistein, 52: Kaempferol, 53: Apigenin, 54: Amentoflavone, 55: Chrysin, 56: Acacetin)

In a study on the effect of seasonal changes (Spring, Summer, Autumn, and Winter) on phenolic compounds in *P. formosum*, chlorogenic acid was reported to be the most abundant phenolic compound. In the same study, gallic acid, protocatechuic acid, 4-OH-bezoic acid, caffeic acid, vanillic acid, *p*-coumaric acid, and ferulic acid were detected in different amounts. Except ferulic acid, all the detected components are compatible with the current study (Rajčić *et al.* 2023). The presence of quinic acid is an advantage because in a recent study (Benali *et al.* 2022). It was shown that the compound has antioxidant, antidiabetic, anticancer, antimicrobial, antiviral, anti-aging, cardioprotective, antinociceptive, and analgesic effects. Protocatechuic acid is also a good antioxidant and anti-inflammatory compound, while 4-OH-benzoic acid has antibacterial and antifungal properties and is widely used in various foods (Mao *et al.* 2019).

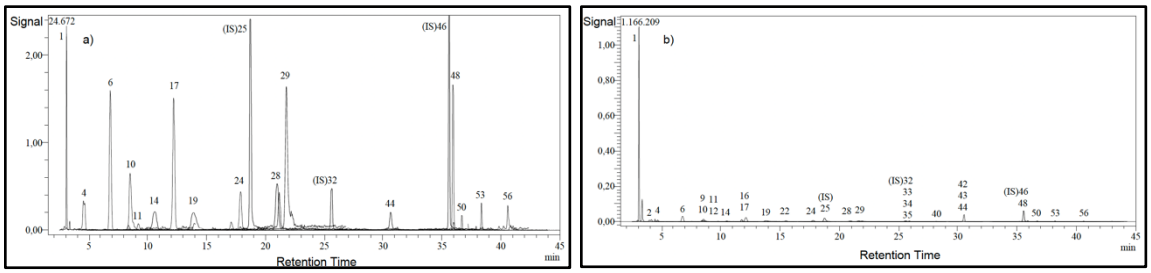

Fig. 4. LC-MS (TIC) chromatogram of *P. formosum* (a), and *P. commune* (b)

p-Coumaric is tyrosinase inhibitor (Boo 2019) and fisetin has neurotrophic, anticarcinogenic, anti-inflammatory, and other beneficial health effects (Khan *et al*. 2013). If quantities of these detected phenolics were compared with those from previous literature (Ertas and Yener 2020) based on other species, these values would be either better or comparable with reported work.

CONCLUSIONS

1. As study on bryophytes was carried out with the goal of expanding the potential of these species, particularly *P. formosum* and *P. commune* from the Polytrichaceae family, as valuable sources of medicinal compounds for the health sector. Detailed investigations into their chemical composition—including metal concentrations, phenolics, and fatty acids—alongside bioactivity studies, suggest that both species have potential as sources of medicinal raw materials.
2. The species *P. formosum* and *P. commune* are underexploited for their valuable biologically active compounds. High concentrations of oleic acid and palmitic acid have been identified. In addition, 12 elements and 28 phenolic compounds were detected.

3. Strong antioxidant activity, in agreement with the identified constituents, underscores the importance of both species and highlights opportunities for further research. It is believed that these species could serve as valuable raw material sources with high potential, especially for the cosmetic and health industries.
4. Comprehensive analyses of these species have demonstrated their high antioxidant capacity, rich profile of phenolics and fatty acids, and abundance of essential minerals. These findings underscore the untapped bioactive potential of bryophytes and pave the way for further research to harness their therapeutic and commercial benefits.

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