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IFE JOURNAL OF SCIENCE AND TECHNOLOGY

Vol 9. No. 1 (2025) 95 – 103

Evaluating the Nutritional Potentials in Selected Bryophyte Species - Proximate, Mineral and Antinutrient Analyses

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Abstract
Selected bryophyte species were investigated with a view to assessing their potential dietary significance. The collected species were thoroughly washed, air dried and analysed for the proximate, mineral and antinutrient contents following standard procedures. The results of the proximate analyses revealed that the moisture content of the species ranged from 7.13 to 11.64% while the ash content ranged from 5.27 to 26.62%. The protein content ranged from 15.45 to 19.02%. Crude fibre content (9.18 to 12.23%), ether extract content (0.69 to 1.71%) and carbohydrate content (39.91 to 51.15%) were also recorded. The elemental analysis showed the presence of macro- and micro- nutrients in the investigated bryophyte species which are valuable inorganic nutrients vital for various physiological functions in animals. All the species were found to contain noticeable levels of phytate and oxalate with the phytate (11.95 to 14.24 mg g^{-1}) being higher than the oxalate contents (2.31 - 3.22 mg g^{-1}). The study established that, bryophytes, though minute in sizes, have the potential to be an alternative nutrient source that could offer nutritional benefits like those of other edible terrestrial plants for both man and animal.

Keywords: Antinutrient factor, bryophytes, feeds, minerals, proximate composition

INTRODUCTION

Bryophytes, comprising mosses, liverworts, and hornworts, are the earliest land plants (Swati *et al*., 2023). They are capable of colonising rock surfaces and initiating the process of soil formation (Anna and Lynn, 2025). Their dense mat growth habits and rhizoids help bind the soil particles, reducing the risk of soil erosion in areas with steep slopes or disturbed soil (Anna and Lynn, 2025). Bryophytes lack vascular tissues but can grow where vascularized plants cannot as they do not depend on root for the uptake of nutrients from the soil. They absorb water and nutrients from their habitats through the surface of their aerial parts such as leaves and stem, but may also be conducted through rhizoids (Glime, 2017). A comparatively stable substratum to attach remains the principal requirement for bryophytes to grow, a platform that retain moisture for long periods, appropriate temperature, enough sunlight and humid atmosphere would be equally appropriate for them to flourish (Ochyra *et al*., 2020; Jukonienė *et al*., 2022).

Plants have been the primary source of food for man and other animals from time immemorial. The increasing growth in human population and the need to develop more new healthy diets require looking for new alternative foods as well as sources of important food ingredients which is in line with the United Nations Sustainable Development Goal 2 (UN-SDG), Zero Hunger, focusing on developing means to address hunger for the growing population of the planet (McKeon-Bennetta and Hodkinson, 2021). In this respect, bryophytes are highly relevant and stand as prospective objects of study. Although usually overlooked in nutritional studies, research have established their potentials as a source of bioactive compounds that are of great importance to man (Olasoji *et al*., 2019; Swati *et al*., 2023). To advance these studies on alternative food sources, it is important to study bryophytes and their nutritional status. The fact that bryophytes are among the most widely distributed plant species in the world and are found relatively in all ecosystems with the exception of saltwater environments (Anustup and Abhijit, 2022) makes it important to investigate their nutritional status to bring these readily available creatures into more good uses.

Analysis of a bryophyte species for nutritional composition involves among others the determination of the macronutrients content such as carbohydrates, proteins and lipids as well as the micronutrients content (Ali *et al*., 2021). The nutritional composition of a plant describes the qualitative as well as the quantitative analyses of the various components present in it (Radha *et al*., 2021) and, having this knowledge can in no small measure have significant impact on the plant's value. Hence in this study, the proximate, elemental and antinutrient constituents of three selected bryophyte species (*Archidium ohioense*, *Hyophila involuta* and *Pelekium gratum*) were investigated. This is believed would assist in assessing the potential and/or the overall dietary significance of the bryophytes.

METHODS

Collection and Preparation of Plant Materials

Three different moss plants were investigated in this study. The mosses are *Archidium ohioense* Schimp ex. Mull, *Hyophila involuta (*Hook) Jaeg., and *Pelekium gratum* (Palis) Jaeg. They were collected from their natural population at the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (Latitude 7° 31' 14.7612" N and Longitude 4° 31' 49.1340" E). Each of the moss samples was collected into a separate paper envelope and taken to the laboratory where it was carefully separated from dirt, washed with distilled water, and air dried. The air-dried samples were ground and then kept in airtight bottles in order to maintain the samples integrity before further laboratory analyses.

Proximate Analyses

The proximate analyses to determine the moisture, crude fat, crude fibre, crude protein, ash and carbohydrate contents of the three bryophytes were carried out using standard procedures (AOAC, 2010).

Elemental Analysis

The air-dried bryophyte samples were analysed for their elemental contents following a previously described standard method (Akinyemi *et al*., 2022) at the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria by flame atomization, using PG990 Atomic Absorption Spectrometer employing air-acetylene flame and single element hollow cathode lamp. The air-dried powdered samples (0.25 g) were further dried in the oven at 45°C and then put into a Teflon beaker. To this was added, twenty (20) ml of freshly prepared aqua-regia, $HCl:HNO_3$ (3:1), heated to near dryness and allowed to cool. Distilled water (20 ml) was added, heated for five (5) min, allowed to cool and filtered. The filtrate was then made up in a 25 ml flask for elemental analysis using Atomic Absorption Spectrometer. Different concentrations of each element were equally prepared from the stock solution (1000 ppm) for each cation and used in preparation of calibration curve for the elemental analyses.

Antinutrient Analysis

Oxalate content determination

The oxalate contents of the bryophyte samples were determined following the method described by Falade (2004). Two (2) g of the air-dried samples were digested with 10 ml 6 M HCl for one hour and each was made up to 250 ml in a volumetric flask. The pH of the filtrates was adjusted with concentrated ammonium hydroxide solution (NH_4OH) until the solution colour changed from salmon pink to a faint yellow colour. It was then treated with 10 ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate (left for 24 h). The suspension was centrifuged at 2500 rpm, after which the supernatant was decanted, and the precipitate was completely dissolved in 10 ml of 20% (v/v) H_2SO_4. The total filtrate resulting from the dissolution in H_2SO_4 was made up to 300 ml. An aliquot of 125 ml of the filtrate was heated until near boiling point and then titrated against 0.05 M of standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titre value. The overall redox reaction is:

1 ml of 0.05M $KMnO_4$ = 2 mg sodium oxalate equivalent per gram of sample

Phytate content determination

Phytate content of the samples was determined using the method of Iwuozor (2019). One (1) g of each sample was hydrated in distilled water (50 ml) for an hour and then filtered. Aliquot of 25 ml of the filtrate in a conical flask was added to 5 ml of 0.30% ammonium thiocyanate, and the mixture was titrated with Iron (III) chloride solution to a persistent brownish yellow colouration that persisted for 4 min. The phytates were then calculated using the formula:

$Phytic\ acid = Titre\ value \times 0.00195 \times 1.9 \times 100$.

RESULTS

Proximate Analysis

The results of the proximate analyses (Table 1) revealed the dry matter contents of the bryophyte species to be generally high, recording the highest percentage values (92.873% in *A. ohioense*, 89.373% in *H. involuta* and 88.363% in *P. gratum*) compared to the other components. This was followed by the carbohydrate content with the values 49.090%, 54.983% and 63.383% in *A. ohioense*, *H. involuta* and *P. gratum* respectively. Ether extract recorded the least percentage contents in all with the value of 1.707% in *A. ohioense*, 0.960% in *H. involuta* and 0.693% in *P. gratum*.

Table 1: Proximate composition of selected bryophyte studied

Proximate Content	*Archidium ohioense*	*Hyophila involuta*	*Pelekium gratum*
Moisture (%)	7.127 ± 0.134	10.627 ± 0.184	11.637 ± 0.137
Ash (%)	26.623 ± 0.235	18.220 ± 0.173	5.267 ± 0.328
Crude Fibre (%)	9.180 ± 0.231	11.107 ± 0.317	12.230 ± 0.139
Ether Extract (%)	1.707 ± 0.145	0.960 ± 0.033	0.693 ± 0.071
Crude Protein (%)	15.453 ± 0.169	15.210 ± 0.139	19.020 ± 0.157
Nitrogen Free Extract (%)	39.910 ± 0.294	43.877 ± 0.228	51.153 ± 0.365
Total Carbohydrate (%)	49.090 ± 0.289	54.983 ± 0.223	63.383 ± 0.328
Dry Matter (%)	92.873 ± 0.134	89.373 ± 0.184	88.363 ± 0.137

Values are means ± standard errors of three replicate determinations

Elemental analysis

The results of the species analyses for sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), cadmium (Cd), cobalt (Co), nickel (Ni), lead (Pb), arsenic (As) and mercury (Hg) contents are presented in Fig. 1. The concentrations of each of the elements in each of the moss species varied. In *A. ohioense,* Na had the highest concentration, followed by K while Hg had the least concentration. In *H. involuta*, the element with the highest concentration was also Na while the As and Hg (each 0.003 ppm) recorded the least. On the other hand, Ca showed the highest concentration in *P. gratum*, while As and Hg (each 0.003 ppm) again recorded the least concentration. *A. ohioense* recorded the higher Na content (2.908 ppm) compared to *H. involuta* (0.990 ppm) and *P. gratum* (1.087 ppm). *P. gratum* had the highest K concentration (5.900 ppm) followed by *A. ohioense* (0.633 ppm) while *H. involuta* recorded the least value (0.507 pmm). Again, *P. gratum* showed the presence of higher concentration of Ca (8.100 ppm), Mg (1.483 ppm), Iron (1.583 ppm), Cu (0.331 ppm), Zn (0.842 ppm), Cr (0.085 ppm) than *A. ohioense* and *H. involuta*. Low concentrations of Cd (0.021 ppm), Co (0.018 ppm), Ni (0.017 ppm) in *P. gratum* were also recorded but still significantly higher than the concentration recorded for *A. ohioense* and *H. involuta*.

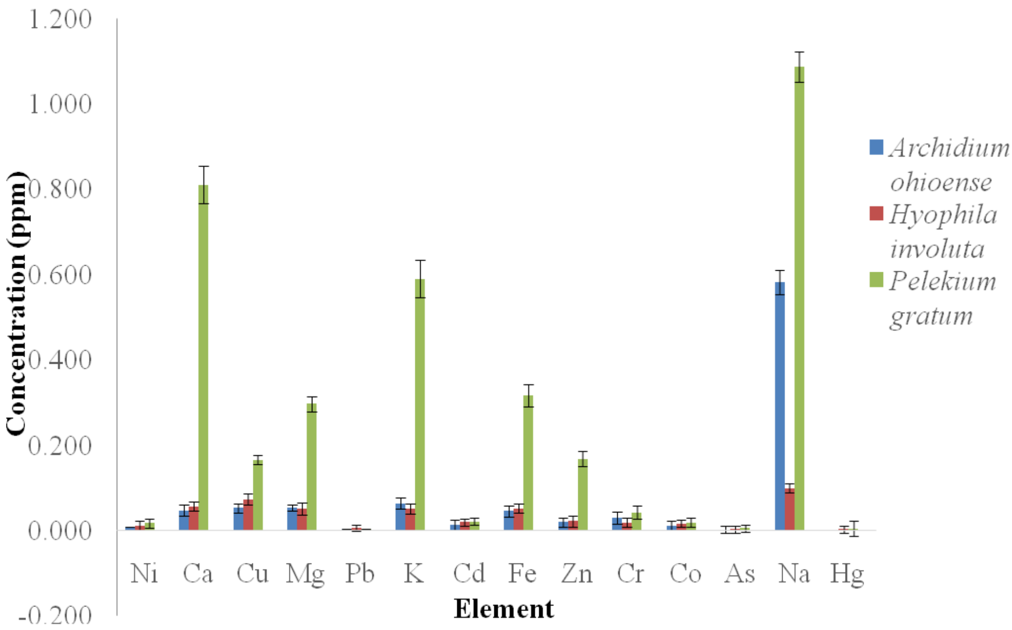

Figure 1: Elemental composition of selected bryophyte species

Antinutrient analysis

The concentration (mg g^{-1}) of the antinutritional factors (phytate and oxalate) in the three investigated bryophyte species; *Archidium ohioense*, *Hyophila involuta*, and *Pelekium gratum* is presented in Table 3 below. *P. gratum* exhibited the highest phytate (14.24 mg g^{-1}) and oxalate (3.22 mg g^{-1}) levels, followed closely by *H. involuta*. Generally, the phytate contents were higher than the oxalate contents.

Table 2: Antinutrient contents of selected bryophytes studied

Moss	Phytate(mg g^{-1})	Oxalate (mg g^{-1})
Archidium ohioense	11.950 ± 0.257	2.307 ± 0.081
Hyophila involuta	14.050 ± 0.107	3.077 ± 0.053
Pelekium gratum	14.237 ± 0.207	3.223 ± 0.087

Values are means ± standard errors of three replicate determinations

DISCUSSION

The results of the proximate analyses of *Archidium ohioense, Hyophila involuta, and Pelekium gratum* in this study revealed the presence of nutritional elements in these species. Although each of the species displayed different nutritional composition profiles, the identified nutritional contents could necessitate their being considered as potential sources of food or feed resources. Proximate analysis of plant samples is important in determining the potential applications of the plants for use in food, medicine, or other industries as it provides useful information about their nutritional composition (Arup *et al.*, 2024), helping in identifying any potential health benefits or risks that could be associated with consuming the plant in question.

The low moisture contents ranging from 7.13% to 11.64% which was consistent with a high dry matter content (89.37% to 92.87%) recorded in the species could be attributed to be one of the factors why bryophytes have good shelf stability and a reduced perishability (Iwuagwu and

Okonkwo 2014). The high dry matter contents recorded in this species indicated a high proportion of structural and functional compounds in the biomass. This makes bryophytes a good candidate for biochemical and nutritional extraction, as dry matter contains most of the organic nutrients including proteins, lipids, and carbohydrates (Adamu *et al*., 2024). Low moisture content of the bryophytes would also encourage a reduced microbial degradation if considered for storage or feed formulation (Kristin *et al*., 2022). High dry matter is also advantageous for biomass processing and quantification in industrial applications such as biofuel production, where water content must be minimized (Demirbas, 2007).

The generally high total carbohydrate contents (49.09% to 63.38%) recorded in this study compared very well with those of some edible terrestrial plants such as black bean seeds (*Phaseolus vulgaris* L.) (58.107%) (Kotue *et al*., 2018), and some species of beans, where 56.80% to 60.57%, carbohydrate contents were reported (Ejimofor *et al*., 2023). The nitrogen-free extract (39.91% to 51.15%) representing the soluble carbohydrates such as sugar and starch contents in the bryophyte species point to their potential as a good energy source, possibly supporting their ecological fitness in nutrient-limited habitats (Marschner, 2012). The percentage values recorded for nitrogen-free extract in the species also indicated that the bryophyte species store and/or are composed of more digestible energy, supporting their potential as carbohydrate-rich biomass. High carbohydrate levels are beneficial for metabolic energy and could support the bryophyte's ecological role in nutrient cycling and energy transfer in its habitat (Kristin *et al*., 2022). Carbohydrates in bryophytes also serve as vital reserves for regrowth following desiccation (Sabovljević and Sabovljević, 2008).

The crude fibre contents (9.18% to 12.23%) in the studied species are of nutritional importance. Fibres have been reported to be good in aiding digestion and enhancing gastrointestinal function in herbivores, underscoring the bryophytes' potential applications in forage or feed formulations (Rao and Sharma 1997; Lu *et al*., 2005; Arup *et al*., 2024), and ecologically significant for water retention in bryophyte colonies (Glime, 2007). The crude fibre contents indicate the amount of indigestible cellulose present in the species and the values recorded reflect the structural component and stable cell wall architecture they possess. Crude fibre is important for maintaining bryophytes integrity and resistance to mechanical and environmental stress (Glime, 2007). In dietary terms, crude fibre promotes gut motility and prevention of constipation but reduces digestible energy (Barber et *al*., 2020). The relatively moderate fibre contents recorded in the studied bryophytes stressed the potential in the species as fibre sources, if included in dietary formulations where fiber is desired. The percentages of the ether extract components were low across the three analysed bryophyte species suggesting that the species are not substantial fat sources. This observation agrees with Sabovljevic *et al*. (2016) who reported that low-fat content is a common trait in non-vascular cryptogamic plants. Although, it has been reported that even in low quantities, bryophyte lipids may include physiologically relevant compounds such as essential fatty acids and fat-soluble vitamins which may have antimicrobial or medicinal properties (Sabovljević *et al*., 2016), and as well play important roles in cellular functions (Yi, *et al*., 2019). The presence of crude protein (15.45% to 19.02%) in the studied bryophyte species suggests that the species could serve as a relative source of protein and nitrogenous compounds, making them a potential candidate for alternative protein sources in livestock feed formulations or biofertilizers. Protein is crucial for cellular repair, enzyme synthesis, and overall growth and development of humans and other animals (Elango and Laviano 2017; Ali *et al*., 2021).

The percentage ash content of the studied bryophyte species indicated the proportion of the total mineral matters in them and revealed their mineral accumulation potential. Bryophytes are known to accumulate nutrients from their surroundings due to their high surface area-to-volume ratio and lack of a cuticle, making them a strong candidate for biomonitoring applications or mineral recovery (Tyler, 1990; Ares *et al*., 2012). The high ash contents documented in this study demonstrates that the selected bryophytes may serve as mineral supplements. Several studies have

investigated the mineral composition of different plant species (Yi *et al*., 2019; Arup *et al*., 2024). The presence of the essential nutrient elements such as Ca, Na, K, Mg, Zn, Fe, Pb, Cu each of which are vital for various physiological functions in man and animals indicates that the bryophytes can be a valuable source of the inorganic nutrients (Aberoumand, 2011). The low but detectable levels of heavy metals like Ni, Pb, Cd, Co, As and Hg in the species highlighted the potential of these bryophytes as bioindicators of heavy metal pollution in an environment, aligning with the reported studies on bryophyte biomonitoring potentials (Drobnik and Stebel, 2021).
Antinutritional factors (phytate and oxalate) in the three bryophyte species were reported for their protective ecological roles, including herbivory deterrence and ion chelation in the bryophyte species (Glime, 2007). These antinutritional substances are natural compounds known for their interference with the bioavailability of essential mineral nutrients such as Ca, Mg, Fe, and Zn present in plants by forming insoluble complexes (Oladayo *et al*., 2019). Phytate as an example has the ability to form insoluble complexes with minerals, primarily iron, zinc, and calcium, thereby reducing their bioavailability and the eventual nutrient deficiencies (Akinyemi *et al*., 2022). Oxalate was also reported to form calcium oxalate deposit in plant tissues (Oladayo *et al*., 2019). However, the levels of the oxalate contents recorded for the bryophyte species in this work were lower than the toxic thresholds of 4.5 mg/g reported in higher plants (Erdman, 1979; Akwaowo *et al*. 2000). Although, antinutritional factors could negatively affect nutrients bioavailability, food processing methods such as drying, boiling, roasting or fermentation have been reported to mitigate their effects (Upreti and Dixit, 2009; Opega *et al*., 2016), thus allaying the fear of antinutrient presence in a plant once they are identified. However, further investigations into the digestibility, biochemical and toxicological capacities of the species are necessary to fully exploit their potentials as a novel feed, bio-resource, and other industrial applications.

CONCLUSION

The three studied bryophyte species; *Archidium ohioense, Hyophila involuta* and *Pelekium gratum* showed the potential to serve as an alternative source that could offer nutritional benefits to animals like those of other edible terrestrial plants. The species exhibited high dry matter contents and are rich in carbohydrates and protein as compared to other proximate contents. The high carbohydrate contents suggested that the bryophytes are potentially good source of energy. The relatively high ash contents together with the various nutrient elements analysed in the species signify that they are rich in minerals. The antinutrients; oxalate and phytate were present but in low amount.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria for providing the enabling environment to carry out this study, and to Professor A.M. Makinde of Botany Department, Obafemi Awolowo University Ile-Ife, Osun State, Nigeria for his advice and linguistic contribution to the work.

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