

Phytochemical and proximate compositions of frond extracts of *Nephrolepis biserrata*, *Phymatosorus scolopendria*, and *Microgramma mauritiana* in Rivers State University, Nigeria

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Abstract. Ibiye A, Green BO, Ajuru MG, Chikere LC. 2024. Phytochemical and proximate compositions of frond extracts of *Nephrolepis biserrata*, *Phymatosorus scolopendria*, and *Microgramma mauritiana* in Rivers State University, Nigeria. *Asian J Nat Prod Biochem* 22: 1-7. Pteridophytes have a great range of forms and are cosmopolitan in distribution. There are about 13,500 species of fern and allies distributed throughout the world. This research was carried out to investigate the phytochemical and proximate compositions of three species of ferns growing at Rivers State University, Nigeria. The three fern species were *Nephrolepis biserrata* (Sw.) Desv., *Phymatosorus scolopendria* (Burm.fil.) Pic.Serm., and *Microgramma mauritiana* (Willd.) Tardieu. The phytochemical and proximate composition analyses were done using standard procedures. Results from the phytochemical analysis showed that glycoside was the highest content in the three ferns, that is, *P. scolopendria* (17.24%), *N. biserrata* (14.47%), *M. mauritiana* (12.8%), followed by alkaloid in *M. mauritiana* (7.18%), *N. biserrata* (6.10%), *P. scolopendria* (4.42%). Phenol content in *M. mauritiana*, *P. scolopendria*, and *N. biserrata* were 4.09%, 2.17%, and 1.33%, respectively. Flavonoid content in *N. biserrata*, *P. scolopendria*, and *M. mauritiana* were 3.16%, 2.87%, and 2.43%, respectively. Saponin content in *P. scolopendria*, *M. mauritiana*, and *N. biserrata* were 1.65%, 1.35%, and 1.29%, respectively. Tannins content in *P. scolopendria*, *M. mauritiana*, and *N. biserrata* were 2.54%, 1.91%, and 0.98%, respectively. Results from proximate analysis showed that the highest moisture content was in *M. mauritiana* (53.39%), followed by *P. scolopendria* (50.78%) and *N. biserrata* (44.58%). The lowest content in the frond extracts was ash, with the values of *M. mauritiana* (0.84%), *N. biserrata* (0.83%), and *P. scolopendria* (0.73%). The results of the phytochemical and proximate evaluation of the species studied indicated that they are nutritionally and medicinally important, and their consumption can provide essential nutrients humans need.

Keywords: *Microgramma mauritiana*, *Nephrolepis biserrata*, *Phymatosorus scolopendria*, phytochemicals, proximate analysis

INTRODUCTION

Pteridophytes are non-flowering vascular cryptogamic plants. Pteridophytes comprise 400 genera and approximately 10,500 species, including living and fossil plants. Ferns are vascular plants with xylem and phloem bundles, which enable them to transport water from the root to other parts of the plant (xylem) and to translocate food to different plant parts (phloem). Most ferns are leptosporangiate, producing coiled fiddleheads that uncoil and develop into fronds. There are about 10,560 known living species of ferns, and they are generally grouped as Polypodiopsida, which comprises both the leptosporangiate (Polypodiidae) and eusporangiate ferns.

Nephrolepis biserrata (Sw.) Desv. plants are mainly used for ornamental purposes. In Sarawak, it is used to treat wounds, blisters, abscesses, and boils on the skin. In India, the rhizomes are used to treat respiratory diseases. It is also used to prevent miscarriage, promote fetal development, and treat stomach pain, bleeding, and wounds (Piggott 1996; Malan and Neuba 2011). Due to its high nutritional value, it serves as fodder for African dwarf goats (Christensen 1997). The tip of young shoots is used as a vegetable by the indigenous people of Malaysia.

Phymatosorus scolopendria (Burm.fil.) Pic.Serm. frond is pounded in Indo-China and used to treat boils and filariasis. Whole fronds are kept on beds to ward off bed bugs, while the young fronds are used to treat chronic diarrhea. In Polynesia, the fronds are ground and mixed with scrapings of *Atuna racemosa* Raf. to produce perfume. Also, the mashed fronds are wrapped with leaves of *Morinda citrifolia* L., then boiled and used as medicinal bandages. Frond juice is used in Fiji to treat stomachaches, swollen breasts, and boils (Snogan et al. 2007). They are also used to treat asthma, cough, and inflammatory diseases.

Microgramma mauritiana (Willd.) Tardieu is mainly found in moist scrubs, short forest patches, and climbing shrubs, and it produces fertile fronds in higher light intensity. Whole plants are used against pubic lice in humans and to prevent the reproduction of the lice.

Phytochemicals or plants' secondary metabolites are often restricted to a particular species within a phylogenetic group. Secondary metabolites are very important in treating diseases and in the ecological interaction of plants with other organisms. Proximate analyses of plant extract include moisture, ash, crude protein, crude lipid, crude fiber, and digestible carbohydrates. Proximate composition analysis is used to determine the nutritional constituents of

plants. It is essential because most medicinal plants are consumed due to their medicinal value; therefore, analyzing their nutrient content could provide added value to these plants (Pandey et al. 2006).

Ferns are plants widely distributed, including Nigeria, with emphasis on Rivers State. Generally, ferns have been classified into different taxonomic hierarchies, though there is very poor or little information on the relationships or differences between several ferns in their taxonomic classification. Most ferns are neglected, while some are endangered due to inadequate information, cultivation, and taxonomic studies. Researchers globally have carried out credible taxonomic works on several fern families. In Nigeria, the information characterizing these ferns is scanty, and the morphological approach seems to be predominant, especially those that are epiphytic and terrestrial. For example, two fern species of *Nephrolepis* have been investigated based on their morphological parameters. The use of biosystematic methods can improve the classification system. This work focused on providing systematic information using the phytochemical and proximate analysis.

MATERIALS AND METHODS

Collection, identification, and preparation of plant materials

The research work took place from July to November 2023. The different fern species were freshly collected from various trees in Rivers State University, Nkpulu-Oroworukwo, Rivers State, Nigeria. The plant samples were assigned accession numbers and identified at the Department of Plant Science and Biotechnology Herbarium, Rivers State University, Nigeria, by a Plant Taxonomist. The plant specimens were deposited in the Herbarium. Fresh fronds from each species were thoroughly washed with distilled water thrice and then dried in a shady place for five days. Dried plant samples were ground to powder using mortar and pestle.

Extraction of plant materials

About 100g of the powdered plant was carefully weighed and loaded into a soxhlet extractor. The powdered materials were extracted separately with redistilled methanol and petroleum spirit (60-80°C) using soxhlet extraction and cold maceration. The extracts were then concentrated in a vacuum using a rotary evaporator at 40°C. Dried extracts were used for further analysis.

Procedures

Proximate analysis

Determination of moisture content

The standard AOAC (2000) method was followed to deduce the Moisture contents. One gram of the sample was placed in a dry, empty, pre-weight (W1) clean petri dish (with lid). Samples were oven-dried at 105°C for 4-5 hours until a constant weight was obtained, and then the sample was placed in a desiccator for 30 minutes. After cooling the dish, the petri dish and sample were weighed as final

weight (W2). The percentage of moisture was calculated as follows:

$$\% \text{Moisture} = \frac{\text{Wt. of residue } (W^2 - W^1)}{\text{Wt of Sample}} \times 100$$

Determination of ash

Ash content was determined according to the AOAC (2000) method. A clean crucible was heated in a muffle furnace for an hour at 660°C, then placed in a desiccator to cool. After cooling, it was weighed (W1). Ten g of dry sample was taken in the crucible. The sample was burned on the burner with the help a blowpipe. The crucible containing the sample was heated at 550°C for 6-8 hours in a muffle furnace. After cooling to room temperature, the crucible was weighed as (W2). Percent ash was calculated as follow:

$$\% \text{Ash} = \frac{\text{Wt. of Ash } (W^2 - W^1)}{\text{Wt of Sample}} \times 100$$

Determination of fat

A thimble with dry sample was prepared and its weight recorded as W1. Soxhlet extractor was used for the extraction, with diethyl ether as the solvent, the thimble inside the thimble holder was clipped, then 40 mL of diethyl ether was added into a round bottom flask to extract fat content. The round bottom flask was attached to the extractor with the ring clamped tightly. The temperature was adjusted to 40°C and the heater, main power and the condenser water were switched on. The extraction process was carried out for 16 hours at condensation rate of 2 to 3 drops per second. After the extraction, the ether was allowed to drain out of the thimble after about 30 minutes. The extract was transferred into a pre-weighed Beaker (W2) for further evaporation at room temperature. Room temperature drying was employed to avoid possible explosion from oven-induced drying of the ether solvent. The weight of Beaker and residue (W3) was recorded on completion of the drying process. Note: Excessive drying may oxidize the fat and give high results.

The remaining residue in thimble was used for fiber analysis and calculated as follows:

$$\% \text{Fat (Crude Lipid)} = \frac{\text{Wt. of Extract } (W^3 - W^2)}{\text{Wt of Sample } (W^1)} \times 100$$

Determination of crude fibre

Crude fiber was determined by acid and alkali digestion using fiber tec apparatus following AOAC (2000). The thimble residue was weighed (W1) and then digested with acid, followed by alkali. Then, 100 mL of 2.5% HCl was added to the sample into a beaker, heated with stirring for about 30 minutes, and drained into the beaker. The residue was redigested in 2.5% NaOH. The residue was transferred to a pre-weighted (W2) dried crucible to remove the moisture. The crucible was kept in the furnace until white and grey ash formed. The crucible was cooled in desiccators and weighed again. (W3) The loss in weight of

the dry residue upon ignition was taken as the amount of crude fiber. The crude fiber percentage was calculated as follows:

$$\% \text{Crude Fibre} = \frac{\text{Wt. of dry residue } (W^3 - W^2)}{\text{Wt of Sample } (W^1)} \times F \times 100$$

Where, F: Value of crude fat; W1: Weight of Sample; W2: Weight of dry crucible; W3: Weight of crucible after heating.

Determination of protein: Kjeldahl method

Stage 1

Digestion. 0.1 grams of the sample, 3 grams of the digestion catalyst, and 20 mL of concentrated sulphuric acid were placed in a 250 mL conical flask and then heated to digest until the mixture inside the conical flask turned black to sky blue. The digest was allowed to cool down to room temperature and then diluted with distilled water to 100 mL.

Stage 2

Distillation. The digested catalyst was measured (20 mL) and deposited into a distillation flask, which was inserted into the electrothermal heater. A Liebig condenser was connected to a receiver with the flask attached. The receiver contained 10 mL of 2% boric acid as an indicator. 40 mL of hydroxide solution was injected into the digest using a syringe attached to the single-arm steelhead until the digest became highly alkaline. Then, the mixture was boiled. Distilled ammonia gas was added to the receiver beaker through the condenser, which changed the color of boric acid from purple to green.

Stage 3

Titration. The distillate was titrated back to purple from green using a standard 0.1N hydrochloric acid solution. The quantity of hydrochloric acid used for the titration was the titer value and calculated as follows:

$$\% \text{Organic nitrogen} = \frac{\text{titer value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times 0.1}$$

The titer value is the HCL volume used to titrate the ammonium distillate. 1.4 is the quantity of Nitrogen equivalent to the volume of HCl used in the titration of 0.1N. 100 is the total volume of digest dilution, while the second 100 is the Percentage factor. 1000 is the conversion factor from gram to milligram, while the value 20 is the integral volume of digits analyzed and 0.1 is the weight of the sample in grams digested.

Screening of carbohydrate. Carbohydrates content was determined by subtracting the weights of protein, fats, crude fibers, ash, and moisture contents from 100.

$$\text{TCH} (\%) = 100 - (\text{CP} + \text{A} + \text{CF} + \text{M})$$

Phytochemical analysis

Qualitative analysis

Determination of Flavonoids

1 g of the extract was dissolved in 1% Aluminum chloride in methanol and then added with a few drops of concentrated HCL, magnesium, and potassium hydroxide solution. Orange to pink color change indicated the presence of flavonoids.

Determination of Alkaloids

1 g of the powdered sample was mixed with 5 mL of 1% HCL in a steam bath. Then, the mixture was filtered. After the filtration, 1 mL of the filtrate was exposed to a few drops of Dragendorff's reagent, which caused the sample to change to black, indicating the presence of alkaloids.

Determination of Saponin

2 g of the sample was boiled in 20 cm³ of distilled water inside a water bath and then filtered. 5 cm³ of the filtrate was mixed with 5 cm³ distilled water and shaken thoroughly. The formation of a stable foam indicates the presence of saponin.

Determination of Phenol

2 mL of extract was added with 2 mL of ferric chloride (FeCl₃) solution in a test tube. A deep bluish-green solution indicated the presence of phenol.

Screening of Terpenoid

1 g of the powdered sample was stirred with 2 mL chloroform and 3 mL of conc. H₂SO₄ to form a layer. A reddish-brown interface indicated the presence of terpenoids.

Screening of Tannins

10 mL of distilled water was mixed with 1 g of sample in a test tube, heated, and then filtered. A few drops of 5% ferric chloride were added to the filtrate. A black color indicated the presence of tannins (Banso and Adeyemo 2006).

Screening of Cyanogenic glycosides

3 mL of chloroform and 1 mL of 10% ammonium solution were added to 2 mL of the extract. The formation of a pink color indicated the presence of glycoside.

Quantitative phytochemical evaluation

Alkaloids analysis

Quantitative analysis for alkaloids followed the method by Harborne (1973). 5 g of the sample was placed in a 250 ml Beaker glass. Then, 200 mL of 10% acetic acid in ethanol was mixed with the sample and covered. The mixture was left for 4 hours and then filtered. After filtration, the extract was concentrated in a water bath until one-quarter of the original volume was obtained. Then, concentrated ammonium hydroxide was applied drop by drop until the residue was complete. It was allowed to stand for some time, after which the precipitate was collected and rinsed with dilute ammonium hydroxide before filtering. The residue was alkaloid, and it was dried and weighed. The alkaloid content was calculated as follows:

$$\text{Alkaloid}(\%) = \frac{\text{Filtered paper with residue} - \text{weight of dried filtered paper}}{\text{Weight of Sample}} \times 100$$

Determination of Flavonoids

Determination of quantitative flavonoids followed the method by Kumaran and Karunakaran (2006). The method is based on the formation of flavonoids-ammonium complex, which has an absorptivity maximum of 415 nm. 100 μ L of the extracts in methanol (10 mg/mL) was mixed with 100 μ L of 20% aluminum trichloride in methanol and a drop of acetic acid. The mixture was diluted with methanol to 5 mL. The absorption was observed at 415 nm and read after 40 min. The blank sample was prepared by adding a drop of acetic acid and added with methanol to 5 mL. Under the same conditions, the absorption of standard rutin solution (0.5 mg/mL) in methanol was measured. It was determined in triplicates.

$$\text{Flavonoid(\%)} = \frac{\text{Flask with residue} - \text{weight of empty flask}}{\text{Weight of Sample}} \times 100$$

Determination of Saponin

Obadoni and Ochuko's (2001) method was used to determine saponin quantitatively. 20 g plant powder was put into a conical flask and added with 100 ml of 20% aqueous ethanol. The samples were heated in a hot water bath (55°C) for 4 hours and stirred continuously. Then, it was filtered. The residue was re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL using a hot water bath at about 90°C. The concentrate was taken, placed into a 250 mL separator funnel, added with 20 mL of diethyl ether, and shaken vigorously. The ether layer was discarded while recovering the aqueous layer. This purification process was repeated, and 60 mL of n-butanol was added. The extracts were rinsed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. The samples were dried in the oven to a constant weight after evaporation. Saponin content was calculated as follows:

$$\text{Saponin(\%)} = \frac{\text{weight of conical flask with residue} - \text{weight of empty conical flask}}{\text{Weight of Sample}} \times 100$$

Determination of Phenol

1 g of the powdered sample was weighed and poured into a conical flask and added with 10 mL of ethanol. Then, the flask was sealed with aluminum foil. After shaking it vigorously, the mixture was left to stand for 30 min for proper extraction. The mixture was centrifuged to obtain a clear supernatant to determine the total phenolic content quantitatively.

$$\text{Phenol content mg/kg (TAE)} = \frac{\text{Conc. obtained in mg/l} \times \text{volume of sample} \times \text{DF}}{\text{Sample weight}}$$

DF: Dilution factor, if not diluted, then DF = 1

Determination of Terpenoid

0.4 g of the sample was placed into a conical flask, added with 9 mL ethanol, and macerated for 24 hours. After maceration, the mixture was filtered using a funnel, filter paper, and a conical flask. 20 mL of petroleum ether was employed for the extraction with a separating funnel, then 10 mL of distilled water was added to the extract. The

plant sample was then added to a pre-weighed moisture can and dried in the oven for 1 hour at 150°C for complete dryness. Calculation:

$$\text{Terpenoid} = \text{Can} + \text{Solvent} - \text{Can weight}$$

$$\text{Terpenoid(\%)} = \frac{\text{Total Terpenoid}}{\text{Sample weight}} \times 100$$

Determination of Tannins

500 mg of the sample was placed into a 50 mL plastic bottle, added with 50 mL of distilled water, and shaken for an hour in a mechanical shaker. It was then filtered into a 50 mL volumetric flask and added to the mark with distilled water. Afterward, 5 mL of filtrate was pipetted into a test tube and mixed with 2 mL of 0.1M FeCl₃ in 0.1N, HCL, and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm in 10 minutes and calculated as follows:

$$\text{Tannin (mg/kg)} = \frac{\text{The gradient of tannin} \times \text{absorbance of sample} \times 100}{2}$$

Determination of Cyanogenic glycosides

Five grams of the sample was placed into a clean distillation flask, added with 20 mL of distilled water, and allowed to stand overnight for proper hydrolysis. The sample was distilled using 200 mL of 0.5g NaOH pellets/ 1 L distilled water (AOAC 2000). The distillate was titrated with 0.02 N Silver Nitrate, 5% Potassium iodide, and 1 mL of 6 N Ammonia hydroxide solution to permanent turbidity, which indicated the endpoint.

Cyanogenic glycoside in the sample was calculated as follows: 1 mL 0.02N AgNO₃ = 1.08 mg HCN.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 25 was used to analyze data, and the results were expressed as the mean \pm Standard Error (SE).

RESULTS AND DISCUSSION

Proximate analysis

The result of the proximate analysis of the studied ferns is presented in Table 1. The highest moisture content was in *M. mauritiana* (53.39 \pm 0.16), followed by *P. scolopendria* (50.78 \pm 0.13) and *N. biserrata* (44.58 \pm 0.25). Protein content in *N. biserrata* was (35.56 \pm 0.68), followed by *M. mauritiana* (31.20 \pm 0.05) and *P. scolopendria* (28.12 \pm 0.09). Fibre content in *P. scolopendria* is (11.25 \pm 0.06), *N. biserrata* (8.07 \pm 0.07), *M. mauritiana* (6.23 \pm 0.13). Carbohydrate content in *N. biserrata* (8.29 \pm 0.48), *P. scolopendria* (7.83 \pm 0.12), *M. mauritiana* (6.59 \pm 0.26). Crude Lipid in *N. biserrata* was (2.65 \pm 0.08), *M. mauritiana* (1.76 \pm 0.06), *P. scolopendria* (1.34 \pm 0.03), and ash content in *M. mauritiana* was (0.84 \pm 0.03), *N. biserrata* (0.83 \pm 0.03), *P. scolopendria* (0.73 \pm 0.04).

Phytochemical composition

Qualitative phytochemical analysis of three ferns species

The result of the qualitative phytochemical analysis is presented in Table 2. Glycosides were the highest (++) in all three fern species, followed by Saponin (+), Flavonoid (+), Alkaloids (+), Tannins (+), and Phenols (+).

Quantitative phytochemical analysis of three ferns species studied

The result of the quantitative phytochemical analysis is presented in Table 3. Glycoside content was the highest in the three ferns, i.e., *P. scolopendria* (17.24±0.13), *N. biserrata* (14.47±0.26), *M. mauritiana* (12.8±0.18), followed by Alkaloid in *M. mauritiana* (7.18±0.18), *N. biserrata* (6.10±0.20), *P. scolopendria* (4.42±0.16), the next was Phenol in *M. mauritiana* (4.09±0.08), *P. scolopendria* (2.17±0.04), *N. biserrata* (1.33±0.77), next to Flavonoid in *N. biserrata* (3.16±0.19), *P. scolopendria* (2.87±0.04), *M. mauritiana* (2.43±0.02), Saponin in *P. scolopendria* (1.65±0.03), *M. mauritiana* (1.35±0.04), *N. biserrata* (1.29±0.08) and Tannins in *P. scolopendria* (2.54±0.09), *M. mauritiana* (1.91±0.02), *N. biserrata* (0.98±0.11).

Table 1. Proximate compositions in the fronds of the studied ferns

Parameter (%)	<i>Nephrolepis biserrata</i>	<i>Phymatosorus scolopendria</i>	<i>Microgramma mauritiana</i>
Moisture Conten	44.58±0.25	50.78±0.13	53.39±0.16
Carbohydrate	8.29±0.48	7.83±0.12	6.59±0.26
Crude lipid	2.65±0.08	1.34±0.03	1.76±0.06
Proteins	35.56±0.68	28.12±0.09	31.20±0.05
Fibre	8.07±0.07	11.25±0.06	6.23±0.13
Ash	0.83±0.03	0.73±0.04	0.84±0.03

Note: Mean (±) SD, n=3

Table 2. Qualitative phytochemical constituents of the ferns

Phytochemical	<i>Nephrolepis biserrata</i>	<i>Phymatosorus scolopendria</i>	<i>Microgramma mauritiana</i>
Saponin	+	+	+
Flavonoid	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Glycosides	++	++	++

Note: +: Present; ++: Highly present

Table 3. Quantitative phytochemical constituents of the ferns

Phytochemical (%)	<i>Nephrolepis biserrata</i>	<i>Phymatosorus scolopendria</i>	<i>Microgramma mauritiana</i>
Saponin	1.29±0.08	1.65±0.03	1.35±0.04
Flavonoid	3.16±0.19	2.87±0.04	2.43±0.02
Alkaloids	6.10±0.20	4.42±0.16	7.18±0.18
Tannins	0.98±0.11	2.54±0.09	1.91±0.02
Phenols	1.33±0.77	2.17±0.04	4.09±0.08
Glycosides	14.47±0.26	17.24±0.13	12.18±0.18

Note: Mean (±) SD, n=3

Discussion

The proximate analysis of the three species showed that the fern has a high moisture content. The high moisture content of these fern species results in the high activity of water-soluble enzymes and co-enzymes needed for metabolic activities. It helps their leaves stay in a dry environment for an extended period.

The protein content of three fern species ranged from 28-35%. Plants with more than 12% protein are considered good sources of protein. Therefore, the fern species in this study are a good protein source and can be used as supplements in protein-deficient diets. It is comparable to the daily protein requirement of 23-65 g (Chaney 2006). Proteins in the body are used to produce hormones, enzymes, and blood plasma. Proteins increase immunity and can help cell division and growth. Proteins are also needed to replace dead tissues, energy supply, and amino acid source (Igile et al. 2013).

Fibre content in three ferns ranged from 6.23%-8.07% in the fern species. According to Boutwell (1998), dietary fiber could slow down the rate of glucose absorption into the bloodstream, thereby reducing the risk of hyperglycemia. They also reduce plasma cholesterol levels and prevent colon cancer and cardiovascular diseases. The fiber in plants aids digestion, softens stool, and prevents constipation.

Carbohydrate content in the three fern species ranged from 6.59%-8.29%. The presence of carbohydrates in ferns shows they could be a good energy source. Carbohydrates are by-products of photosynthetic processes. It is used as the primary source of energy. Carbohydrates are hydrolyzed in the body to produce glucose, which can be utilized immediately or stored as glycogen in the muscles and liver (Raven et al. 2005).

Crude Lipid in *N. biserrata* was 2.65±0.08%, 1.76±0.06% in *M. mauritiana*, and 1.34±0.03% in *P. scolopendria*. The results showed that ferns in this study have a low content of crude lipids and can be easily incorporated into a weight-reducing diet. Dietary fats are important because of their high energy value and the soluble vitamins and essential fatty acids in natural foods' fat. Dutta (2003) reported that fats and oil help regulate blood pressure and play a role in synthesizing and repairing vital cell parts.

Ash content in the three ferns in this study was low. Ash content is an indicator of the total mineral content of a sample. Minerals can be used to establish and maintain the acid-alkaline balance of the blood system (Vunchi et al. 2011).

The results of the phytochemical analysis in three fern species are presented in Table 3. Glycoside was the highest compound in the three ferns, i.e., *P. scolopendria* was 17.24±0.13%, 14.47±0.26% in *N. biserrata*, and 12.8±0.18% in *M. mauritiana*. Glycosides are natural toxins found in plants (Walker et al. 2000) and, if consumed in excess, can cause chronic and acute health problems (Singh and Upadhyay 2012; Khoja et al. 2022; Murthy et al. 2023).

Alkaloid content in *M. mauritiana* was 7.18±0.18%, *N. biserrata* was 6.10±0.20%, and in *P. scolopendria* was 4.42±0.16%. Alkaloids are naturally occurring organic compounds found in plants with various pharmacological

activities, including anti-malaria, anti-cancer, and anti-asthma (Kittakoop et al. 2014). Other alkaloids possess psychotropic and stimulant activities, while some can be toxic. A high alkaloid content may be used as an antimalarial, anti-cancerous, and anti-asthmatic (Bandyopadhyay and Dey 2022).

Phenol content in *M. mauritiana* was $4.09 \pm 0.08\%$, in *P. scolopendria* was $2.17 \pm 0.04\%$, and in *N. biserrata* was $1.33 \pm 0.77\%$. Phenolic compounds are natural, large, and complex substances in plants. Phenolic compounds possess defense functions. They display several properties that are quite beneficial to man, including antioxidant properties to protect plants against free radical-mediated diseases. They also perform a defensive role against pathogens, parasites, and predators and provide colors to plants (Walton et al. 2003; Priti et al. 2021).

Flavonoid content in *N. biserrata* was $3.16 \pm 0.19\%$, in *P. scolopendria* was $2.87 \pm 0.04\%$, and in *M. mauritiana* was $2.43 \pm 0.02\%$. The presence of flavonoids in the fronds of these ferns makes them possible to be used in food, medicine, and cosmetics because flavonoids have anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties in addition to their ability to modulate key cellular enzyme functions. Flavonoids in plants are responsible for the color and aroma of flowers and fruits to attract pollinators (Griesbach 2005; Antonisamy et al. 2023).

Saponin in *P. scolopendria* was $1.65 \pm 0.03\%$, in *M. mauritiana* was $1.35 \pm 0.04\%$, and in *N. biserrata* was $1.29 \pm 0.08\%$. Saponin in the fern species showed that it can be used as a surfactant with the potential ability to interact with cell membranes (Lorent et al. 2014). Saponins can be utilized as adjuvants in vaccine development, soap making, medicine, fire extinguishers, dietary supplements, producing steroids and carbonated beverages, anti-feedants, and protecting plants against microbes and fungi (Sun et al. 2009).

Tannin in *P. scolopendria* was $2.54 \pm 0.09\%$, in *M. mauritiana* was $1.91 \pm 0.02\%$, and in *N. biserrata* was $0.98 \pm 0.11\%$. Tannins play a role in protecting against predators and help regulate plant growth (Ferrell 2006). McGee (2004) showed that the astringency from tannin can cause a dry and pucker feeling in the mouth following consumption.

In conclusion, the results of the proximate evaluation of *P. scolopendria*, *M. mauritiana*, and *N. biserrata* support the utilization of the fronds as food supplements. It also showed that they have good nutritional value. They also possess antioxidant activity, which may have potential in the pharmaceutical industry.

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