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Phytonutrient Composition of *Bryophyllum pinnatum* "(Lam.) Oken" Aqueous Leaf Extracts

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Abstract

Herbs have been used in folk and ethno-veterinary medicine, long before their therapeutic effects were scientifically linked to bioactive phytochemicals. This study was conducted to compare the phytonutrient compositions of unfiltered Bryophyllum pinnatum aqueous leaf extracts (UFBPALE) and filtered Bryophyllum pinnatum aqueous leaf extracts (FBPALE). Fresh Bryophyllum pinnatum *leaves of about 60 g were blended with 300 mL of water for three (3) minutes. The extract was divided into two portions: the first portion, UFBPALE, was stored in a sterile bottle without further processing, while the second portion, FBPALE, was filtered through Whatman No. 1 filter paper before being stored in a sterile bottle. Both extracts were subsequently analyzed in the laboratory for their phyto-constituents. Data were analyzed using SPSS Version 20.0 and expressed as Mean ± SD of three replicates determinations while independent t-test was used to test for the significance. The proximate results showed a significant difference ($p<0.05$) in lipids, ash and crude fibre while phenol and alkaloids in the phytochemical composition were significantly different ($p<0.05$) with UFBPALE containing higher levels of phenols (9.80 g/100g) and alkaloids (8.40 g/100 g) than FBPALE, which had 9.10 g/100g and 7.10 g/100 g, respectively. The amino acid profile shows no significant differences with the exception of arginine, alanine, aspartic acid and threonine. Conversely, all the vitamins and mineral were significantly different ($p<0.05$) except zinc. Vitamins B_1, B_2, B_3, and C were higher in FBPALE than in UFBPALE while the minerals were higher in UFBPALE with the exception of potassium. The findings showed that despite minor differences in composition, unfiltered and filtered* Bryophyllum pinnatum *extracts contain comparable phyto-nutrients and both have potentials for use in livestock production.*

Keywords: *Phytogenics, phyto-constituents, extract, Bryophyllum pinnatum*

Introduction

Phytogenics have gained widespread recognition as effective natural alternatives to antibiotics in livestock production, offering solutions for disease prevention, treatment, and growth promotion. The extensive use of antibiotics in animal husbandry has raised major public health concerns due to the presence of antibiotic residues in animal products such as meat, eggs, and milk contributing significantly to the global challenge of antibiotic resistance in humans. These growing concerns have prompted the exploration and adoption of safer, plant-based alternatives like phytogenics. Several phytogenic plants, including turmeric (Abdaljaleel, 2012), Negro pepper (Ukanwoko & Uhumuavbi, 2020), scent leaf (Olumide and Akintola, 2018), ginger (Khan *et al., 2012*), garlic (Olumide & Odunowo, 2019), bullock (Olumide *et al.,* 2022), and clove (Ayodele *et al.,* 2021), have demonstrated beneficial effects on animal health and performance. Among these, *Bryophyllum pinnatum* stands out for its remarkable nutritional and therapeutic potential.

Bryophyllum pinnatum, a member of the Crassulaceae family (commonly known as the stonecrop or orpine family), is native to tropical regions such as Madagascar, Jamaica, and parts of Africa (Latif *et al.*, 2019). In Nigeria, it is popularly known as "Abamoda" among the Yoruba, "Oda opue" or "Alupu" among the Igbo, and "Shuka halinka" or "Karan masallachi" among the Hausa. Globally, it is recognized by various names reflecting its healing reputation, such as life plant, miracle leaf, resurrection plant, wonder of the world, and good luck leaf. This perennial herb can reach up to 3 meters in height and is characterized by its smooth, opposite leaves. It is known for its slightly sour taste, hot potency, and sweet post-digestive effect. *Bryophyllum pinnatum* is rich in bioactive compounds including proteins, fibres, lipids, and various phytochemicals that contribute to its pharmacological properties (Ogidi *et al.,* 2019*;* Okonwu *et al.,* 2020). Given its promising composition and medicinal value, this study was conducted to identify the best form to utilize *Bryophyllum pinnatum* aqueous leaf extracts in livestock production by comparing the phytonutrient profiles of unfiltered and filtered extracts as there is limited information on this comparison, with the aim of assessing potential variations in their nutritional and phytochemical compositions.

Materials and Methods

Sourcing and Preparation of experimental material

Fresh *Bryophyllum pinnatum* leaves (BPL) were collected from the surroundings of Babcock University, located within the Ilisan community, and authenticated by a qualified botanist from the university. The leaves were carefully detached from the stems, thoroughly rinsed with clean water to remove dirt and debris, and subsequently drained in a clean basket to remove excess moisture.

Preparation of extracts

Sixty grams (60 g) of fresh *Bryophyllum pinnatum* leaves (BPL) weighed with a Camry sensitive scale were blended with 300 mL of water for three minutes using a Qasa blender. The extract was then divided into two portions. The first portion was poured directly into a sterile bottle, while the second portion was filtered once through Whatman filter paper No. 1, following the procedure described by Olumide *et al.* (2022), and subsequently transferred into another sterile bottle. Both extracts were refrigerated and subsequently taken to the laboratory for proximate composition, phytochemical constituents, vitamins, minerals, and amino acid profiles analyses.

Phytonutrient Analysis

Proximate analysis

The proximate composition of *Bryophyllum pinnatum* leaf extract was determined using the methods of Association of Official Analytical Chemists (AOAC, 2010). The dry matter content was determined by oven-drying a measured portion of the homogenized sample at 105°C until a constant weight was achieved. Total ash content was analyzed by incinerating the extract in a muffle furnace at 550°C. Fat content was estimated through extraction with petroleum ether using a Soxhlet apparatus. Crude protein was determined using the Kjeldahl method, while total soluble carbohydrates were calculated by subtracting the combined percentages of ash, crude fibre, crude protein, and crude fat from 100% of the sample's dry weight.

Amino acid profile analysis

Amino acid preparation and analysis were performed using a 616/626 High-Performance Liquid Chromatography (HPLC) system, following the procedure described by Natsir *et al*. (2019).

Vitamins analysis and Mineral analysis

The vitamin contents of the extracts were determined using standard spectrophotometric methods as described by AOAC (1995), except for vitamin C, which was analyzed using the titrimetric method outlined by Okwu (2004). Sodium and potassium concentrations were measured using flame photometry with an X-ray fluorescence spectrophotometer (Philips PW 1210 diffractometer). The samples were aspirated into a JENWAY Flame Photometer (Model PFP7) using filters specific to each mineral element, following the procedure of Oshodi (1992). Phosphorus content was determined using the vanadomolybdate method (AOAC, 1995), while other mineral elements were quantified after wet digestion with an Atomic Absorption Spectrophotometer (BUCK 210 VGP model).

Phytochemicals analysis

Standard spectrophotometric and titrimetric methods were employed to determine the phytochemical constituents of the *Bryophyllum pinnatum* leaf extracts. Flavonoid content was analyzed following the procedure of Boham and Kocipai (1994), while saponins, phenols, and glycosides were quantified using the methods described by Obadoni and Ochuko (2001). Alkaloid content was determined according to Harborne (1973), and tannins were analyzed using the titrimetric method outlined by Onwuka (2005).

Statistical Analysis

Data were analyzed using SPSS Version 20.0. Results were expressed as Mean ± SD of three replicates determinations and independent t-test used to test for the statistical significance of the variations in the composition.

Results and Discussions

Proximate composition

The proximate composition of UFBPALE and FBPALE as illustrated in Figure 1. It revealed that despite having close mean values, the lipids ($p<0.05$), ash ($p<0.01$) and crude fibre ($p<0.05$) in UFBPALE and FBPALE show significant differences between them. Both extracts showed comparable levels of moisture (12.22% and 12.52%), crude protein (15.59% and 15.68%), lipids (5.20% and 5.43%), and crude fibre (9.85% and 9.53%) contents, respectively. However, UFBPALE (19.78%) was higher than the value in FBPALE (17.60%). This difference may be due to insoluble particulate residues retained in the unfiltered extract, contributing to the elevated ash content. The results of this study were consistent with the findings of Okonwu *et al.* (2020), who reported similar proximate composition values for fresh *Bryophyllum pinnatum* leaves, including moisture (9.41%), crude protein (10.42%), ash (4.68%), crude fibre (4.30%), and crude fat (1.55%). Conversely, Ogidi *et al.* (2019) documented lower values for the same parameters e.g. moisture (3.13%), ash (1.81%), protein (3.79%), lipids (0.73%), and crude fibre (0.73%). Likewise, Aja *et al.* (2014) reported protein (1.61%), crude fibre (0.95%), crude fat (1.15%), and ash (0.80%), further illustrating the variability observed in the proximate composition of the plant.

These variations among studies could be influenced by several factors, including differences in extraction procedures, cultivar types, agronomic practices, and environmental conditions such as season and location of cultivation (Kumar *et al.,* 2017; Gautam *et al.,* 2019). This result suggested that *Bryophyllum pinnatum* holds considerable promise as a natural feed additive in poultry nutrition due to its rich nutrient composition, which compares favorably with other established phytogenics such as garlic (Olumide and Odunowo, 2019), bullock *(*Olumide *et al.,* 2022*)*, papaya leaf (Akintunde *et al.,* 2021b*)*, turmeric, and clove (Adebisi *et al.,* 2021*)*. Its balanced levels of protein, fibre, lipids, and other essential nutrients make it a valuable ingredient for enhancing livestock growth and productivity (González-Alvarado *et al.,* 2007). The observed moisture content reflects the plant's tendency toward quick spoilage if not properly processed, while the ash content signifies the presence of important inorganic minerals such as calcium, zinc, magnesium, copper, and potassium. Additionally, the crude protein, crude fibre, and lipid contents could contribute directly to improved feed efficiency, body weight gain, and overall animal performance in animal trials.

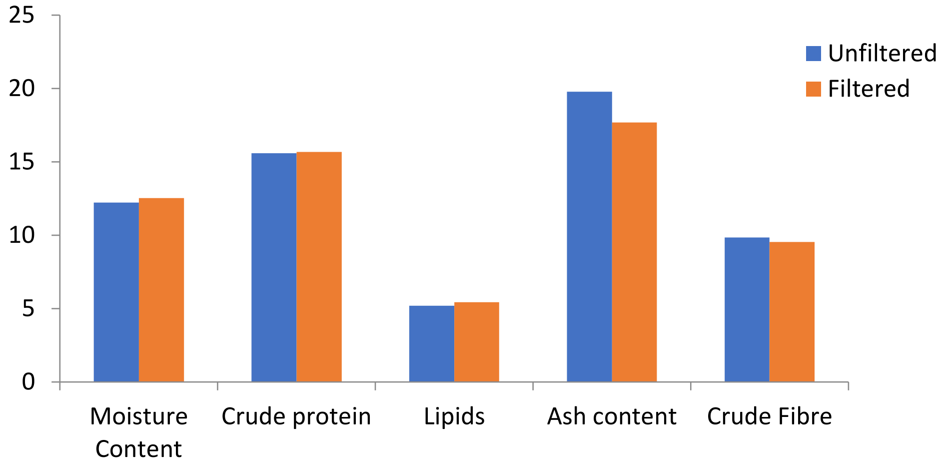

Figure 1: Proximate composition of *Bryophyllum pinnatum* leaf extract

Amino acid profile

The amino acid composition of the unfiltered (UFBPALE) and filtered (FBPALE) *Bryophyllum pinnatum* aqueous leaf extracts is shown in Figure 2. The results revealed that both extracts contained comparable levels of essential and non-essential amino acids, indicating that filtration had little effect on their amino acid profile with the exception of alanine, arginine, aspartic acid and threonine which are significantly different ($p<0.05$). Among the amino acids detected, glycine and threonine were the most abundant, with concentrations of 14.14% and 13.28% in UFBPALE, and 15.68% and 14.08% in FBPALE, respectively. Other amino acids including valine (1.37%), methionine (0.23%), leucine (1.91%), phenylalanine (1.53%), lysine (1.91%), histidine (0.67%), isoleucine (1.18%), arginine (1.77%), serine (1.03%), aspartic acid (1.43%), glutamic acid (2.25%), alanine (2.89%), tyrosine (2.59%), and proline (1.20%) were present in moderate concentrations, with only minor differences observed between the two extracts.

This study revealed higher amino acid values compared to those reported by Okonwu *et al.* (2020), who observed lower concentrations in the leaves and roots of *B. pinnatum*. This variation may be due to differences in extraction methods, which can significantly affect amino acid yield. Furthermore, the amino acid profile observed in this study aligned with the findings from other phytogenic plants such as bitter leaf, moringa, and ginger (Arowora *et al.*, 2017*;* Natsir *et al.*, 2019), underscoring *Bryophyllum pinnatum* as a potential source of high-quality amino acids suitable for livestock nutrition. The amino acid profile of *Bryophyllum pinnatum* aqueous leaf extract (BPALE) highlights its potential as a contributor to dietary protein in livestock.

Glycine, one of the most abundant amino acids in the extract, plays a vital role in protein synthesis for tissue repair and hormone regulation. It also promotes heart and liver health, improves sleep quality, helps regulate blood sugar, and prevents muscle loss when adequately supplied (Razak *et al.*, 2017). Threonine, another key amino acid, serves as a precursor to serine and glycine and is crucial for digestion, immune system function, muscle development, wound healing, and the prevention of fatty liver (Ruggeri, 2019). These benefits align with the traditional medicinal uses of *B. pinnatum*, where the plant has long been valued for its healing properties. Phenylalanine supports the nervous system and acts as a precursor for melanin, the pigment responsible for skin coloration, while tyrosine aids in the production of neurotransmitters such as dopamine and norepinephrine (Rees, 2021). Lysine contributes to hormone and enzyme synthesis, leucine facilitates tissue repair and skin and bone healing, and isoleucine plays a role in hemoglobin formation (Akram *et al.*, 2011). These amino acids collectively underscore the nutritional and physiological importance of *Bryophyllum pinnatum* as a valuable natural supplement capable of enhancing livestock health, growth, and productivity.

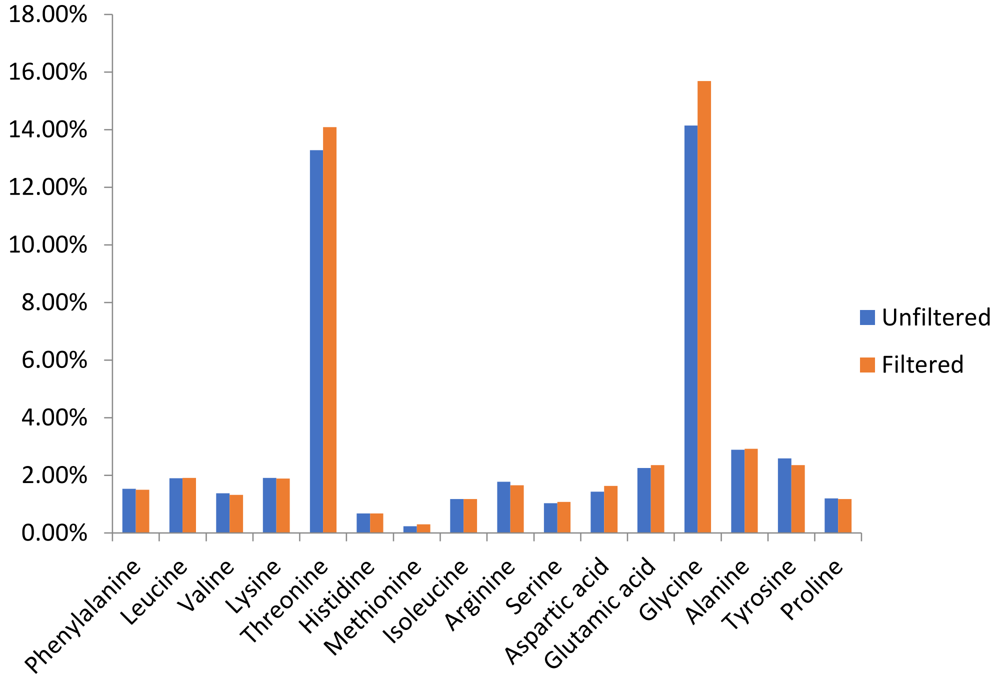

Figure 2: Amino acid composition of *Bryophyllum pinnatum* leaf extract

Vitamin composition

The vitamin composition of the unfiltered and filtered *Bryophyllum pinnatum* aqueous leaf extracts (UFBPALE and FBPALE) is shown in Figure 3. The results revealed the presence of both water-soluble and fat-soluble vitamins in both extracts which are all significantly different in both extracts ($p<0.05$). In UFBPALE, the concentrations of Vitamins A, B_1, B_2, B_3, C, and E were 8.18 mg/100 g, 2.06 mg/100 g, 18.05 mg/100 g, 6.47 mg/100 g, 17.35 mg/100 g, and 19.5 mg/100 g, respectively. In comparison, the corresponding values in FBPALE were 6.63 mg/100 g, 2.57 mg/100 g, 20.0 mg/100 g, 8.3 mg/100 g, 23.5 mg/100 g, and 12.0 mg/100 g. The higher concentrations of water-soluble vitamins (B_1, B_2, B_3, and C) in FBPALE can be attributed to the filtration process, which removes insoluble particles such as fibres, cell debris, and other solid residues. This process concentrates the soluble nutrients in the filtrate. Conversely, fat-soluble vitamins (A and E) showed lower values in FBPALE, as filtration tends to remove particulate-bound or insoluble vitamin forms, leaving behind mainly the soluble fraction. The UFBPALE, however, retains both soluble and insoluble components, accounting for its higher fat-soluble vitamin levels.

According to Okonwu *et al.* (2020), *Bryophyllum pinnatum* leaves contained folic acid (Vitamin B9) as the most abundant vitamin. In comparison, the concentrations of

Vitamins A and E observed in this study were lower than those reported by Okonwu *et al.* (2020), who recorded 11.61 mg/100 g and 70 mg/100 g, respectively, for *Bryophyllum pinnatum* leaves. However, the present values are higher than those reported by Olumide *et al.* (2022) for *Parquetina nigrescens* leaf extract, which contained 2.27 mg/100 g of Vitamin A and 0.0015 mg/100 g of Vitamin E. Similarly, the concentrations of water-soluble vitamins (B_1, B_2, and B_3) obtained in this study were higher than those reported by Okwu and Josiah (2006), who found 0.18 mg/100 g, 0.42 mg/100 g, and 0.02 mg/100 g, respectively. However, they were lower than the much higher values (50 mg/100 g, 130 mg/100 g, and 360 mg/100 g) reported by Okonwu et al. (2020) for *Bryophyllum pinnatum* leaves. The concentration of vitamin C recorded in this study is lower than 58.9 mg/100 g reported by Sharma and Chauhan (2018) for *Bryophyllum pinnatum* leaves. It is also lower than the values reported for other phytogenic plants such as *Andrographis paniculata* (59.5 mg/100 g) and *Clitoria ternatea* (65.8 mg/100 g), both of which are comparable to *Bryophyllum pinnatum* in nutritional potential.

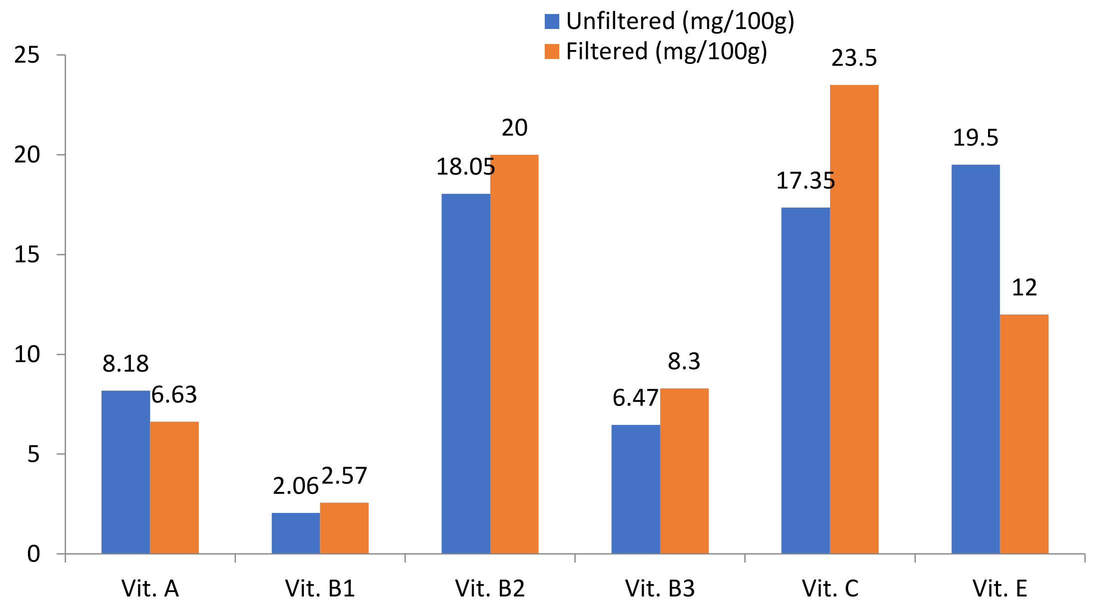

Figure 3: Vitamin composition of *Bryophyllum pinnatum* aqueous leaf extract

Mineral composition

Figure 4 presents the mineral composition of the UFBPALE and FBPALE. Among the macronutrients, calcium showed the highest concentration in both extracts, with values of 410 mg/100 g in UFBPALE and 379 mg/100 g in FBPALE. Potassium followed as the second most abundant mineral, recorded at 231 mg/100 g in UFBPALE and 260 mg/100 g in FBPALE. Magnesium and phosphorus were also present in considerable amounts, with magnesium measuring 39 mg/100 g in UFBPALE and 35 mg/100 g in FBPALE, while phosphorus values were 67.5 mg/100 g and 61.5 mg/100 g, respectively with all except zinc being significantly difference ($p<0.05$) in both extracts. In contrast, Sharma and Chauhan (2018) reported much higher concentrations for potassium (1771.8 mg/100 g) and phosphorus (412.0 mg/100 g) in *Bryophyllum pinnatum* leaves. Similarly, Ogidi *et al.* (2019) observed varying levels, with potassium at 171.7 mg/100 g, calcium at 384.7 mg/100 g, magnesium at

185.3 mg/100 g, and sodium at 297.8 mg/100 g, which differ from the findings of this study.

This study also revealed the presence of key micronutrients, with copper being the least concentrated, followed by zinc and iron. Ogidi *et al.* (2019) reported copper (1.2 mg/100 g), zinc (0.74 mg/100 g), and manganese (2.0 mg/100 g) in *Bryophyllum pinnatum*, whereas Okonwu *et al.* (2020) documented substantially lower values of less than 0.0008mg/100g for copper, zinc, iron, and manganese. These mineral elements play vital roles in metabolic and physiological processes. Calcium and phosphorus are essential for bone development and structural integrity, magnesium contributes to enzyme activation, potassium supports nerve and muscle function, and trace minerals like iron, zinc, copper, and manganese are critical for blood formation, oxygen transport, immune response, and antioxidant defense. The mineral profile of *Bryophyllum pinnatum* aqueous leaf extract highlights its nutritional and therapeutic potential, reinforcing its value as a potential supplement in livestock feeding and natural medicine while its wound healing and blood clotting potential is likely linked to its high calcium content.

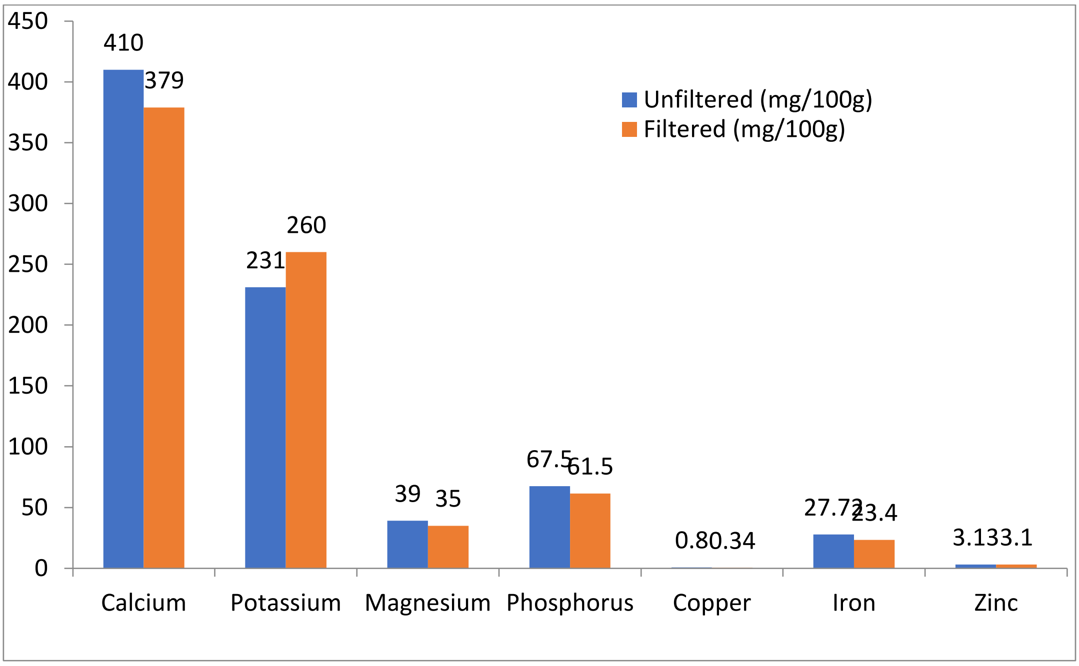

Figure 4: Mineral composition of *Bryophyllum pinnatum* aqueous leaf extract

Phytochemical composition

The phytochemical analysis of UFBPALE and FBPALE revealed significant differences ($p<0.05$) in phenol and alkaloid content while others show no significant differences. Both UFBPALE and FBPALE showed similar phytochemical profiles, with notable concentrations of phenols, flavonoids, and alkaloids. The phenols (9.80g/100g), flavonoids (0.80g/100g), and alkaloids (8.40g/100g,) content found in UFBPALE were slightly higher than the values 9.10g/100g, 0.70g/100g, and 7.10g/100g, respectively recorded in FBPALE. Tannins, saponins, and phytosteroids were present in moderate quantities: 0.18g/100g, 0.49g/100g, and 0.22g/100g, respectively, in UFBPALE, compared with the values (0.13g/100g, 0.49g/100g, and 0.21g/100g) obtained in FBPALE. These findings aligned with the results of Ogidi *et al.* (2019), who reported that *Bryophyllum pinnatum* leaves contained the highest concentration of phytochemicals compared to its roots and stems. Ogidi *et al.* (2019) and Okonwu *et al.* (2020) reported 10.48g/100g and 7.437g/100g respectively for phenols in *Bryophyllum pinnatum* leaves thereby corroborating these findings.

Alkaloids, another significant bioactive compound, was also present in substantial amounts (7.10g/100g in FBPALE). Phenols are secondary metabolites essential to plant physiology, playing key roles in growth, structural integrity, defense, pigmentation, and lignification (Kumar *et al.*, 2014). They are known for their diverse physiological properties, including anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardio-protective, and vasodilatory effects (Kumar *et al.*, 2014). Alkaloids are known for their therapeutic properties, including analgesic, antispasmodic, and bactericidal effects (Ogidi *et al.*, 2019). Tannins, unique polyphenolic compounds with high molecular weight, are widely found in plants and are known for their antioxidant, antimicrobial, antiviral, and cardio-protective properties (Serrano *et al.*, 2009). Flavonoids**,** another group of natural phenolic compounds, are recognized for their essential roles in pharmaceutical, nutraceutical, and medical applications (Panche *et al.*, 2016).

Saponins are known for their ability to precipitate and coagulate red blood cells as well as, their haemolytic and cholesterol-binding properties (Okonwu *et al.*, 2020), The presence of saponins in *Bryophyllum pinnatum* suggested that it may possess these bioactive properties. Phytosteroids, naturally occurring plant steroids, include compounds such as progesterone, phytoandrogens, and phytocorticoids. These substances can interfere with steroid-metabolizing enzymes, influencing the endocrine and reproductive systems (Dean *et al.*, 2017; Hugh *et al.*, 2013). The presence of these beneficial phytochemicals further positioned *Bryophyllum pinnatum* as a valuable resource for its antioxidant and antimicrobial potentials. The presence of phytosteroids in *Bryophyllum pinnatum* also highlights its potential role in supporting growth and reproduction.

Table 1: Quantitative and Qualitative phytochemical composition of *Bryophyllum pinnatum* aqueous leaf extract

Parameters	UFBPALE		FBPALE	
	Qual.	Quant. (g/100g)	Qual.	Quant.(g/100g)
Phenol	+ + +	9.80 ± 0.14[a]	+ + +	9.10 ± 0.28[b]
Flavonoids	+ + +	0.8 ± 0.14	+ + +	0.70 ± 0.01
Alkaloids	+ + +	8.40 ± 0.09[a]	+ + +	7.10 ± 0.28[b]
Tannins	+ +	0.08 ± 0.00	+ +	0.13 ± 0.04
Saponin	+ +	0.49 ± 0.05	+ +	0.49 ± 0.14
Phytosteroids	+ +	0.22 ± 0.01	+ +	0.21 ± 0.01

**p<0.05 +++ = present in high concentration ++ = moderate concentration + = low concentratiom

Conclusion

Based on the results of this study, both unfiltered and filtered *Bryophyllum pinnatum* aqueous leaf extracts (UFBPALE and FBPALE) contain proteins, fibre, lipids, inorganic matter (ash), vitamins, and minerals and beneficial phytochemicals indicating that the leaf has the potential to contribute to the nutritional requirements of livestock. Although, the unfiltered *Bryophyllum pinnatum* aqueous leaf extract (UFBPALE) generally contains significantly higher levels of phytonutrients with the exception of water-soluble vitamins which is anticipated since it retains both soluble and insoluble constituents. However, filtration neither causes significant nutrient loss nor excessively concentrates the filtered extract. Both extracts display a composition similar to other phytogenics, underscoring the potential of *Bryophyllum pinnatum* as a valuable resource for livestock production. Additionally, the presence of phytochemicals in *Bryophyllum pinnatum* suggests its medicinal properties, including antimicrobial, anti-inflammatory, and wound-healing properties.

Conflict of interest
This research work has no conflict of interest.

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