Available online at sjuoz.uoz.edu.krd



Science Journal of University of Zakho Vol. 12, No.3, pp.388–395 July-September, 2024



p-ISSN: 2663-628X e-ISSN: 2663-6298

EVALUATION OF PROXIMATE, PHYTOCHEMICAL AND MINERAL COMPOSITIONS OF LEAF, STEM AND ROOT OF *BRYOPHYLLUM PINNATUM* AND *PHYLLANTHUS AMARUS*

Olayinka, B.U., ^{1*} Babatunde, M. O. ¹Ogundare, G. O.¹, Kayode, O. V.² Muhammad, T. H.³ Daramola, G. G.,⁴ Bulala, A.F¹ & Abdulbaki, S.A^{5&6}

¹Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin.

²Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan.

³Faculty of Science, National Open University of Nigeria, Ilorin Study Centre.

³Department of Plant Biology, Faculty of Pure and Applied Sciences, Kwara State University, Malete.

⁴ Department of Biochemistry, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Osun State, Nigeria.

⁵ Department of Plant Sciences and Biotechnology, Faculty of Life Sciences, Federal University, Dutsin-Ma, Katsina State.

⁶Department of Biological Sciences, Faculty of Sciences, King Abdulaziz, University, Jeddah,

Saudi Arabia.

*Corresponding author's email address: olayinka.bu@unilorin.edu.ng

Received: 18 Jul., 2024 / Accepted: 8 Aug., 2024 / Published: 28 Aug., 2024. <u>https://doi.org/10.25271/sjuoz.2024.12.3.1338</u>

ABSTRACT:

Bryophyllum pinnatum and *Phyllanthus amarus* are plants that have been established to contain bioactive substances for various therapeutic advantages, most importantly their leaves. Information on the other parts of the plant, such as stem and root, is scanty in literature; hence, there is a need to compare the various parts of the plants in terms of proximate composition, phytochemicals, and mineral elements. The results showed that the two plants had the highest amount of carbohydrate (62.09 - 63.98%) and followed in decreasing order of magnitude by protein (16.04 - 17.50%), moisture (10.05 - 10.35%), fibre (3.9 - 4.36%) and ash (3.80 - 4.03%). The fat contents in both plants ranged from 1.09% to 2.03% in *Phyllanthus amarus* and *Bryophyylum pinnate* leaves, respectively. Alkaloids (106.97 - 108.46 mg/100g) were highest in all the plants, while steroids (0.11 - 0.17 mg/100g) were lowest. Generally, in both plant parts, the leaf and stem had a higher amount of the foregoing proximate and phytochemicals when compared to the root. Considering the macronutrients, all the plant parts contained the highest amount of potassium (29.04 - 30.03 mg/100g) and the lowest amount of nitrogen (0.25 - 0.27%). Iron (26.3 - 27.7 mg/100g) was the highest among the micronutrients, while copper was the lowest (0.70 - 0.73 mg/100g). The study concluded that both plants showed proximate phytochemicals and mineral nutrients that were unevenly distributed in the various parts of the plant, with the leaf and stem exhibiting higher concentrations compared to the root. Therefore, adequate knowledge of the distribution of these chemical constituents will provide a baseline for the selection of parts to be used for health benefits.

KEYWORDS: Proximate, phytochemical, mineral composition, Phyllanthus amarus, Bryophyylum pinnatum.

1. INTRODUCTION

The use of medicinal plants dates back thousands of years. Available evidence suggests the use of plants as medicines may go as far back as 60,000 years ago (Hosseinzadeh *et al.*, 2015). However, the vast majority have not been studied for biological activity, and even fewer have undergone phytochemical analysis (Salmerón-Manzano *et al.*, 2020; Agidew, 2022). This only opens up a vast area for further research on the efficacy of plants as medicine.

The *Bryophyllum pinnatum*, popularly known as 'Never die', is another economically important succulent perennial plant used in traditional medication due to its diverse phytochemical content. The plant is native to Madagascar and has been found in regions of Asia, Australia, New Zealand, Africa, and America (Chibli *et al.*, 2014). It is also employed to cure various diseases like inflammation and diabetes (Ogidi *et*

al., 2018). In Nigeria, it is even used by traditional birth attendants, and it is used to help release the placenta of any newborn baby (Ogidi et al., 2018). Methanolic leaf extracts enhance wound healing by suppression of inflammation as well as up-regulation of vascular endothelial growth factor (VEGF) mediated angiogenesis (Araújo et al., 2023). It has also been found to have a strong anti-inflammatory ability in both shortand long-term inflammation models (Chibli et al., 2014). Furthermore, the aqueous leaf extract has great antinociceptive and analgesic effects similar to that of non-steroidal antiinflammatory drugs (NSAIDs) and a noteworthy effect of causing a reduction in blood glucose levels in the diabetic model (Ojewole, 2005). Furthermore, the extracts also possess antioxidant activities that help combat oxidative stress and antimicrobial properties, especially against Helicobacter pylori, as reported by Mabeku et al. (2016). The occurrence of compounds such as flavonoids, phenolic acids, triterpenoids, and bufadienolides make the leaves possess medicinal

This is an open access under a CC BY-NC-SA 4.0 license (https://creativecommons.org/licenses/by-nc-sa/4.0/)

^{*} Corresponding author

properties, as highlighted by other researchers (Fürer *et al.*, 2016; Thorat *et al.*, 2018; Fenandes, 2021).

Phyllanthus amarus is a small herb plant of the Euphorbiaceae family, which contains nearly 800 species (Chinchansuree *et al.*, 2024). The plant's flower, fruit, and leaf seem as though they are one, and this is why the name *'Phyllantus'* can be translated to mean 'leaf and flowers'. It grows extensively as a weed in West African cultivated areas and has been reported from the Philippines, Cuba and India, where it is also common as a weed (Moshi *et al.*, 1997; Verma *et al.*, 2014).

Phyllanthus amarusis is widely known to have medicinal and curing properties for various diseases, including respiratory disorders such as asthma, according to the research work of Pius et al. (2015). Alongside this, the herb shows hepatoprotective properties because it minimizes liver injury caused by toxins such as aflatoxin B1 and paracetamol through antioxidant activities. The herb also increases the levels of both the enzyme and non-enzyme antioxidants in the liver (Nazz, 2007; Mishra, 2013). Also, the ethanolic extracts of the leaves and seeds of Phyllanthus amarus have been used in the treatment of nephrotoxicity caused by acetaminophen and gentamycin through its antioxidant activity with free radical-scavenging properties (Adeneye & Benebo, 2008; Chopade et al., 2021). Its usefulness also includes anti-inflammatory, antiallergic, antiviral, antibacterial, and antimicrobial properties (Yao et al., 2017; Rani et al., 2021). Yao et al. (2017) described its potential role in cardioprotection in hypertensive rats. The anticancer ability of the plant is attributable to the plant's phytochemical content, which includes lignans, flavonoids, and polyphenols (Gupta & Vaghela, 2019). Phyllanthus amarus, therefore, provides a perfect example of a medicinal plant with numerous health benefits in both traditional and modern systems of medicine.

The main objectives of this study are to compare the proximate composition, phytochemicals, and mineral elements in different parts (leaves, stems, and roots) of *Bryophyllum pinnatum* and *Phyllanthus amarus* plants and to determine the distribution of these chemical constituents in the various parts of the plants to provide a baseline for selection of parts to be used for health benefits. This study aims to fill the gap in the literature by providing information on the chemical composition of different parts of these medicinal plants beyond just the leaves. Understanding the distribution of these chemical constituents in the various plant parts will provide additional information to guide the selection of appropriate parts to be used for the various healing properties and aid in scaling up such parts in their use in modern medicine.

2. MATERIALS AND METHOD

2.1 Collection, Identification, and preparation of plant samples

Whole plant specimens of *Bryophyllum pinnatum* and *Phyllanthus amarus* were collected from the University of Ilorin Botanical Garden on the 5th of February, 2024. The specimens were carefully uprooted from the soils. The plants were identified and authenticated at the Herbarium Unit of the Department of Plant Biology at the University of Ilorin. The samples were washed 2-3 times in running water to remove soil particles. Samples were carefully separated into leaves, stems, and roots. They were air-dried and later oven-dried at 40^oC for one hour. Each plant part was blended in a blender into homogenous powder, transferred into sample bottles, and labelled.

2.2 Extraction of samples of plant parts for *Bryophyllum* pinetum and *Phyllanthus amarus*

A quantity of 10 g of the ground sample for each plant part was soaked in 50 ml of methanol in a separate container and left at room temperature for three days (72 hours). The extracts were passed through cotton wool after being filtered using Whatman filter paper No. 42 (125mm). The extracts were evaporated to dryness in a hot water bath for 72 hours and then refrigerated for later use.

2.3 Proximate composition

2.3.1 Moisture Content:

The AOAC (2000) was followed to determine the moisture content. An empty dish and lid were dried for each plant part for three hours at 105 degrees Celsius in the oven before being moved to a desiccator to cool. The empty dish and its lid were weighed. After that, around 3 g of the sample was placed evenly on the dish. The plate containing the sample was put in the oven and dried at 105°C for three hours. The partially covered plate was placed in the desiccator to cool down after drying. The plate and the dried sample were reweighed.

$$Moisture(\%) = \left(\frac{W1 - W2}{W1}\right) * 100$$

Where W1= Weight of the sample before drying W2= Weight of sample after drying

2.3.2 Ash content:

This was determined by incinerating 5.0 g of well-mixed ground sample in a muffle furnace at 600°C for 3 hours until light-grey ash was produced. The percentage of ash content was estimated using the following calculation:

Ash content (%) =
$$\frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.3.3 Crude Fibre: This was determined following the method of the Food and Agriculture Organization

(FAO) (1980). The percentage of crude fibre was calculated as follows:

Crude fibre (%) =
$$\frac{\text{Weight of dry residue}}{\text{Weight of sample}} \times 100$$

Crude Fat: This was achieved by extraction procedure 2.3.4 according to the method of the Federation of Oils, Seeds and Fats Association (FOSFA) (1982). The percentage crude fat content was estimated using the calculation below:

Crude fat (%) =
$$\frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

2.3.5 Crude Protein: This was determined by using the Kjeldahl method (Lindner & Harley, 1942). The

Crude protein (%) =
$$\frac{T \times N \times D \times 0.014}{M}$$

Where T is the volume of the HCl used corrected for blank; N is the normality of the HCl solution; D is the dilution of the final extract 0.014 is obtained from the division of the molecular weight of nitrogen (14) by 1000 (1 litre); C.F. is the factor (6.25) used for converting nitrogen into crude protein, and M is the mass of the sample used.

2.3.6 Carbohydrate Determination:

The carbohydrate content of each sample was determined by adding up the whole proximate analysis and subtracting it from a hundred. 100 - (% protein + % moisture + % ash + % fat + % fiber).

2.3.7 Energy:

The calorific value in each sample was calculated by multiplying the percentage of protein and carbohydrate by 4 and the fat by 9.

2.4 Mineral Quantification

In a digestion glass tube, 0.5 g of dried material was combined with 10 millilitres (10 ml) of H₂SO₄. The final mixture was left to stand at room temperature for the whole night. Subsequently, the mixture was mixed with 4.0 ml of perchloric acid (HClO₄) and stored in a fume cupboard. The temperature of the digestion unit was raised step by step, from 50°C to 300°C. White vapours appeared, indicating that the digestion was completed in around 70-80 minutes. After cooling, the mixture was poured into 100 ml volumetric flasks, and distilled water was added to bring the content to 100 ml. Mineral determination was done using the filtrate, which was obtained by filtering the resulting solution. The micro Kjeldahl technique was used to determine nitrogen (N). An Atomic Absorption Spectrophotometer (AAS) (Shimadzu, Japan AA-6200) was used to determine iron (Fe), magnesium (Mg), and zinc (Zn). Sodium (Na) and potassium (K) were determined using a Flame Photometer (Bibby Scientific Limited, UK: Model No PFP7) in accordance with Allen's (1989) methodology.

Phytochemical Screening

2.4.1 Total Tannins Content Determination:

Total tannin content was determined using the method employed by Ogidi et al. (2019).

2.4.2 Determination of Saponins:

Test extracts were dissolved in 80% methanol, followed by the addition of 2 ml of vanillin in ethanol and thorough mixing. 2ml of 72% sulphuric acid solution was added, well mixed, and heated on a water bath at 600 degrees Celsius for 10 minutes percentage of crude protein was calculated as follows:

ade protein (%) =
$$\frac{T \times N \times D \times 0.014 \times C.F}{M} \times 100$$

before measuring absorbance at 544nm against a reagent blank. Diosgenin equivalents are employed in the test and Diosgenin is used as a standard material (Owoyale, et al., 2019).

2.4.3 Determination of Steroid:

A 100 ml beaker was filled with a 0.05 g sample of extract, and 20 ml of a chloroform-methanol (2:1) mixture was added to the extract. The mixture was shaken for half an hour to remove any remaining steroids. A 30 ml test tube was filled with 1 ml of the filtrate and 5 ml of alcoholic KOH and carefully shaken to create a homogeneous mixture. After that, the combination spent ninety minutes in a water bath with a temperature range of 37°C to 40°C. After cooling it to room temperature, 10 millilitres of petroleum ether and 5 millilitres of distilled water were added. In the water bath, this evaporated to a dry state. 6 ml of Liebermann-Burchard reagent was added to the residue in a dry bottle, and the absorbance was then measured at a wavelength of 620 nm using a Spectronic 21D digital spectrophotometer (Ameen et al., 2021).

2.4.4 Determination of Flavonoids:

A 1 mL of extract was added to a 10 mL volumetric flask containing 4 mL of distilled water, followed by 0.3 mL of 5% NaNO₂. 0.3 ml of 10% AlCl₃ was added after 5 minutes. After adding 2 millilitres of 1M NaOH at the six-minute mark, the volume was increased to 10 millilitres using distilled water. After thoroughly mixing the solution, the absorbance at 510 nm was measured against the prepared reagent blank. The total flavonoid content was expressed as a percentage (Owoyale et al., 2019).

2.4.5 Determination of Alkaloids:

The method used by Ogidi et al. (2019) was used to determine the alkaloid content. 1g of the powdered sample was mixed with 40 millilitres of 10% acetic acid in ethanol, covered, and left to stand for 4 hours. After that, the filtrate was concentrated to a quarter of its initial volume in a water bath. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. After letting the entire mixture settle, the precipitate was collected, washed with diluted ammonium hydroxide, and filtered. After drying, the residue was weighed.

2.4.6 Determination of Terpenoids:

One gram (1 g) of the extract was filtered after being macerated in 50 ml of ethanol. After slowly adding and mixing 2.5 ml of concentrated H₂SO₄ to 2.5 ml of the filtrate, 2.5 ml of 5% aqueous phosphomolybdic acid solution was added. After the mixture had stood for thirty minutes, five millilitres of ethanol were added. At 700 nm, the absorbance was measured (Ogidi *et al.*, 2019).

2.5 Data Analysis

One-Way Analysis of Variance was used to analyze the data gathered from proximate, phytochemical, and mineral studies (ANOVA). Duncan's Multiple Range Test was used to differentiate the means at a significance level of p < 0.05.

3. RESULTS

3.1 Proximate composition and energy value of Bryophyllum pinnatum and Phyllanthus amarus

The results of the proximate composition and energy value (kcal) in both medicinal plants significantly differed (p<0.05)

by species and plant parts, as indicated in Table 1. Considering the plant parts in *Bryophyllum pinnatum*, significant differences were not recorded for protein, carbohydrate, and energy values. Regardless of the plant parts, carbohydrates had the highest percentage mean value ($63.09\pm0.47\%$), followed by protein ($16.63\pm0.48\%$), moisture ($10.18\pm0.03\%$), fiber ($4.18\pm0.05\%$), ash ($3.97\pm0.19\%$), and fat ($1.97\pm0.26\%$) (Table 1). Carbohydrates were higher in the roots (63.98%) than in the stem and leaf (62.09%, 63.21%). Moisture, ash, and fiber were higher in the stem (10.35%, 3.98%, 4.36%) than in the root and leaf.

The proximate composition and energy value (kcal) of *Phyllanthus amarus* followed a similar trend as recorded for *Bryophyllum pinnatum*. Significant differences were not recorded for protein, carbohydrate, and energy values. However, there were significant differences (p<0.05) between and among the other proximate parameters (moisture, ash, fiber, and fat). Carbohydrates were higher in the leaf (64.47%) than in the stem and root (62.57%, 62.20%). Moisture and ash were higher in the root (10.28%, 4.03%) than in the other plant parts.

| | ······································ | | | | - · J · r · · J · · · · · r · · · · · | | ••••••••••••••••••••••• | |
|------------------------|--|--------------------------|-------------------------|-------------------------|---------------------------------------|------------------------|-------------------------|--------------------------|
| Species(S) | Plant parts (P) | Moisture | Ash | Fibre | Protein % | Fat | Carbohydrate | Energy (Kcal) |
| Bryphyllum pinnatum | Leaf | 10.13±0.03 ^{ab} | 3.88±0.3 ^b | 4.13±0.03 ^b | 16.63±0.01ª | 2.03±0.01ª | 63.21±0.03ª | 283.20±0.15ª |
| | Stem | 10.35±0.15ª | 3.98±0.3ª | 4.36±0.07 ^a | 17.23±0.58ª | 1.99±0.13ª | 62.09±0.61ª | 280.37±0.95ª |
| | Root | 10.05±0.01 ^b | 3.97±0.1ª | 4.06±0.40 ^b | 16.04±1.17 ^a | 1.89±0.13 ^b | 63.98±1.18 ^a | 283.20±1.31ª |
| | Mean | 10.18±0.03 | 3.97±0.19 | 4.18±0.05 | 16.63±0.48 | 1.97±0.26 | 63.09±0.47 | 282.37±0.68 |
| Phyllathus amarus | Leaf | 10.13±0.08 ^b | 4.023±0.33 ^b | 3.94±0.26 ^b | 16.37±1.47ª | 1.09±0.07 ^b | 64.47±1.43ª | 279.19±1.57ª |
| | Stem | 10.07±003 ^b | 3.89±0.001° | 4.02±0.03 ^a | 17.50±0.01ª | 2.03±0.12 ^a | 62.57±0.01 ^a | 282.65±0.05 ^a |
| | Root | 10.28±0.04 ^a | 4.03±6.33 ^b | 3.99±0.01 ^{ab} | 17.50±0.87ª | 2.01±0.01 ^a | 62.20±0.90 ^a | 281.58±1.02 ^a |
| | Mean | 10.16±0.03 | 3.97±0.21 | 3.98±0.02 | 17.13±0.52 | 1.703±0.15 | 63.06±0.60 | 281.14±0.74 |
| S | P-value | 0.752 | 0.02 | <0.0001 | 0.57 | < 0.0001 | 0.97 | 0.27 |
| Р | P-value | 0.03 | 0.001 | 0.0020 | 0.95 | <0.0001 | 0.83 | 0.06 |
| S×P | P-value | 0.032 | < 0.0001 | 0.0001 | 0.520 | < 0.0001 | 0.19 | 0.951 |

Table1: Proximate composition and energy value of leaf, stem and root of Bryophyllum pinnatum and Phyllanthus amarus

Mean values followed by the same letter(s) along the column are statistically similar p<0.05

3.2 Phytochemical Screening

The analysis of phytochemical constituents indicated the presence of tannins, alkaloids, flavonoids, saponins, terpenoids, and steroids in the roots, stems, and leaves of both plant species (Table 2). The phytochemical content varied significantly (p<0.05) between species and plant parts. In *Bryophyllumpinnatum*, alkaloids exhibited the highest mean value (108.03 \pm 0.27) across all plant parts, followed by

terpenoids (23.59 ± 0.08) , tannins (10.82 ± 0.26) , flavonoids (9.99 ± 0.19) , saponins (7.01 ± 0.04) , and steroids (0.17 ± 0.03) (Table 2). Significant differences (p<0.05) in phytochemical content were observed between and among plant parts, except for flavonoids and terpenoids, where no significant differences were noted.

Phyllanthus amarus exhibited a similar trend to *Bryophyllum pinnatum*. Among the plant parts, the levels of tannins, terpenoids, and steroids did not differ significantly. However,

the levels of alkaloids, flavonoids, and saponins varied significantly between and among the plant parts (leaf, root, and stem).

Overall, the phytochemical constituents were higher in *Bryophyllum pinnatum* compared to *Phyllanthus amarus*.

Tannins were lower in the stems of both plants, while terpenoids were lower in the leaves of both plant species. These differences were not statistically significant (p<0.05).

| Species (S) | Plant parts(P) | Tannins | Alkaloids | Flavonoids (mg/100g) | Saponins | Terpenoids | Steroids |
|------------------------|-------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Bryphyllum Pinnatum | Leaf | 11.76±0.001ª | 108.46±0.13ª | 9.99±0.27ª | 7.046±0.67ª | 23.58±0.001ª | 0.11±0.01 ^a |
| | Stem | 10.22±0.14 ^b | 108.67±0.06 ^b | 9.72±0.45 ^a | 7.017±0.26 ^b | 23.62±0.07 ^a | 0.17±0.03 ^b |
| | Root | 10.50±0.42 ^b | 106.97±0.04ª | 10.15±0.45 ^a | 6.97±0.01 ^{ab} | 23.59±0.10 ^a | 0.12±0.03 ^b |
| | Mean | 10.82±0.26 | 108.03±0.27 | 9.99±0.19 | 7.01±0.04 | 23.59±0.08 | 0.17±0.03 |
| Phyllathus amarus | Leaf | 10.78±0.28ª | 108.40±0.01° | 9.72±0.01 ^b | 6.97±0.07 ^b | 23.53±0.47ª | 0.13±0.03 ^a |
| | Stem | 10.36±0.28ª | 105.013±0.17ª | 9.45±0.35 ^b | 7.03±0.03ª | 23.63±0.07ª | 0.12±0.01 ^a |
| | Root | 11.06±0.14 ^a | 106.67±0.23ª | 9.35±0.01ª | 7.02±0.01ª | 23.58±0.07 ^a | 0.13±0.03 ^a |
| | Mean | 10.73±0.15 | 106.69±0.49 | 9.49±0.75 | 6.93±0.07 | 23.58±0.17 | 0.12±.0.07 |
| S | P-value | 0.603 | 0.0001 | 0.0001 | 0.075 | 0.439 | 0.012 |
| Р | P-value | 0.003 | 0.0001 | 0.280 | 0.001 | 0.478 | 0.198 |
| S×P | P-value | 0.052 | 0.0001 | 0.003 | 0.0001 | 0.286 | 0.005 |

| Table 2: Phytochemical | constituents of leaf, stem | and root of Bryphyllum | <i>pinnatum</i> and <i>phyllathus amarus</i> |
|--------------------------|-----------------------------|--------------------------|--|
| 1 dole 2. 1 hytoenenneur | constituents of fear, stern | , and root of Dryphymini | printanti and privitantas antaras |

Mean values followed by the same letter (s) along the column are statistically similar at p<0.05

3.3 Macro-elements

The results for macro elements (N, P, K, and Mg) in the leaves, stems, and roots of the two medicinal plants differed significantly by plant species, plant parts, and their interactions, except for nitrogen (Table 3). Regardless of plant parts, *Bryophyllum pinnatum* exhibited significantly higher levels of potassium and phosphorus compared to *Phyllanthus amarus*. Conversely, *Phyllanthus amarus* had significantly higher levels of magnesium than *Bryophyllum pinnatum*. Among all the

macro elements, potassium had the highest values, ranging from 189.08 to 190.35 mg/100g, followed by magnesium (68.31-68.42 mg/100g) and phosphorus (29.38-29.64 mg/100g). Nitrogen had the lowest values (0.26-0.27 mg/100g) compared to the other macro elements (Table 3). In *Bryophyllum pinnatum*, the leaves and stems generally contained significantly higher amounts of all macro elements compared to the roots (Table 3). However, in *Phyllanthus amarus*, the roots and stems had greater amounts of all macro elements compared to the leaves (Table 3).

| Species (S) | Plant parts (P) | Ν | P mg/100g | K | Mg |
|-------------------------|-----------------|------------------------|-------------------------|--------------------------|--------------------------|
| Bryophyllum Pinnatum | Leaf | 0.27±0.01ª | 30.03±0.01ª | 190.35±0.01 ^b | 68.050±0.29ª |
| | Stem | 0.27±0.02 ^a | 29.85±0.11ª | 190.87±0.03 ^a | 63.92±0.06° |
| | Root | 0.25±0.02ª | 29.04±0.32 ^b | 189.96±0.86° | 67.97±0.04 ^b |
| | Mean | 0.26±0.01 | 29.64±0.16 | 190.35±0.14 | 68.31±0.47 |
| Phyllathus | Leaf | 0.26±0.03ª | 28.97±0.03° | 187.97±0.14° | 67.89±0.01° |
| amarus | Stem | 0.28 ± 0.02^{a} | 29.02±0.06 ^b | 189.12±0.07 ^b | 69.01±0.03 ^a |
| | Root | 0.27±0.01ª | 30.07±0.03ª | 190.12±0.01ª | 68.30±0.057 ^b |
| | Mean | 0.27±0.01 | 29.38±0.17 | 189.08±0.30 | 68.42±0.16 |
| S | p-value | 0.65 | < 0.0001 | < 0.0001 | < 0.0001 |
| Р | p-value | 0.69 | < 0.0001 | < 0.0001 | < 0.0001 |
| S×P | p-value | 0.67 | < 0.0001 | < 0.0001 | < 0.0001 |

Mean values followed by the same letter (s) along the column are statistically similar at p < 0.05

3.4 Micro-elements

The microelement content in the leaves stems, and roots of *Bryophyllum pinnatum* and *Phyllanthus amarus* varied significantly between and among the plant parts (Table 4). Sodium (Na), *Bryophyllum pinnatum* showed values of 7.028 \pm 0.03 in the leaf, 7.027 \pm 0.03 in the stem, and 6.967 \pm 0.08 in the root, with a mean of 7.028 \pm 0.07. *Phyllanthus amarus* had values of 7.10 \pm 0.057 in the leaf, 6.967 \pm 0.082 in the stem, and 7.020 \pm 0.01 in the root, with a mean of 7.022 \pm 0.16 (Table 4). Significant differences are found among plant parts (P<0.0001) and their interactions (P<0.0001) but not between species (P=0.829). Considering the copper (Cu) content, *Bryophyllum pinnatum* recorded values of 0.715 \pm 0.003 in the leaf,

 0.73 ± 0.057 in the stem, and 0.70 ± 0.057 in the root, with a mean of 0.715 ± 0.05 . *Phyllanthus amarus* showed values of 0.625 ± 0.08 in the leaf, 0.64 ± 0.07 in the stem, and 0.67 ± 0.15 in the root, with a mean of 0.65 ± 0.079 . Significant differences are observed between species (P=0.001), among plant parts (p=0.004), and their interactions (p=0.001).

For Iron (Fe), *Bryophyllum pinnatum* had values of 27.02 ± 0.06 in the leaf, 26.767 ± 0.04 in the stem, and 26.737 ± 0.08 in the root, with a mean of 26.821 ± 0.05 . *Phyllanthus amarus* recorded values of 26.67 ± 0.08 in the leaf, 26.87 ± 0.08 in the stem, and 27.07 ± 0.06 in the root, with a mean of 26.84 ± 0.064 . Significant differences exist between species (P=0.014), among plant parts (P=0.001), and their interactions (P=0.001).

| Table 4: Variation in Microe | elements in leaf, stem and | d root of <i>Bryophyllum</i> | pinnatum and Phyllathus amarus |
|------------------------------|----------------------------|------------------------------|--------------------------------|
| | | | |

| Species(S) | Plant parts(P) | Na | Cu | Fe mg/100g | Zn |
|-------------------------|----------------|-------------------------|---------------------------|--------------------------|-------------------------|
| Bryophyllum pinnatum | Leaf | 7.028±0.03 ^c | 0.715±0.003 ^{ab} | 27.02±0.06ª | 5.280±0.06ª |
| | Stem | 7.027±0.03 ^b | 0.73±0.057ª | 26.767±0.04 ^b | 5.300±0.05ª |
| | Root | 6.967±0.08° | 0.70 ± 0.057^{b} | 26.737±0.08 ^b | 5.00±0.02 ^b |
| | Mean | 7.028±0.07 | 0.715±0.05 | 26.8211±0.05 | 5.193±0.05 |
| Phyllathus amarus | Leaf | 7.10±0.057a | 0.625 ± 0.08^{b} | $26.67 \pm 0.08^{\circ}$ | 5.22±0.06 ^b |
| | Stem | 6.967±0.082b | 0.64±0.07 ^{ab} | 26.87±0.08 ^b | 4.97±0.01 ^{ab} |
| | Root | 7.020±0.01ª | 0.67 ± 0.15^{a} | 27.07±0.06 ^a | 5.27±0.08 ^a |
| | Mean | 7.02±0.16 | 0.65±0079 | 26.84±0.064 | 5.14±0.04 |
| S | P-value | 0.829 | 0.001 | 0.014 | 0.001 |
| Р | P-value | 0.0001 | 0.004 | 0.001 | 0.001 |
| S×P | P-value | 0.0001 | 0.001 | 0.001 | 0.077 |

Mean values followed by the same letter(s) along the column are statistically similar at p<0.05

For Zinc (Zn), *Bryophyllum pinnatum* showed values of 5.280 ± 0.06 in the leaf, 5.300 ± 0.05 in the stem, and 5.00 ± 0.02 in the root, with a mean of 5.193 ± 0.05 . *Phyllanthus amarus* has values of 5.22 ± 0.06 in the leaf, 4.97 ± 0.01 in the stem, and 5.27 ± 0.08 in the root, with a mean of 5.14 ± 0.04 . Significant differences are found between species (P=0.001) and among plant parts (P=0.001), but not for their interactions (P=0.077).

4.DISCUSSION

The phytochemical and mineral characterization of Bryophyllum pinnatum and Phyllanthus amarus yield valuable information on their respective health benefits. Each plant contains a plethora of various phytochemicals, which explains their use in traditional medicine (Pius et al., 2015; Ogidiet al., 2018). A phytochemical examination of Bryophyllum pinnatum supports the hypothesis generated by Ogidiet al., 2019, in which alkaloids, tannins, saponins, flavonoids, and terpenoids were detected. However, the result is slightly in contrast with the finding by Ogidi et al., 2019 where flavonoids were reported to be absent in the root. As expected, the study revealed that flavonoids were more concentrated in the root than in the leaf or stem. The phytochemical evaluation of Phyllanthus amarus in this experiment corroborates the phytochemical evaluation done by Ameen et al. 2021 where it was identified that the plant contains alkaloids, tannins, saponin, flavonoids, and terpenoids. However, Ameen et al., 2021 only confirmed their presence in the leaves and roots of *Phyllanthus amarus*. This study did confirm that the phytochemicals are also present in the stem. Because *Bryophyllum pinnatum* has higher phytochemical content than *Phyllanthus amarus*, it suggests *Bryophyllum pinnatum* has more medical benefits than *Phyllanthus amarus*. Flavonoids and saponins also have antioxidant, antiinflammatory, and antimicrobial effects. *Bryophyllum pinnatum* has significant usage in traditional medicines (Kamboj & Saluja, 2009).

Both plants contain high levels of carbohydrates in their tissues and stems and vary in moisture, ash and fibre content depending on the body part of the plant used. The second subgenera, *Bryophyllum pinnatum*, contains higher carbohydrate content in the roots, and the subgenera *Phyllanthus amarus* contains higher carbohydrate content in the leaves. Experiments reveal that there is high moisture and ash content in the stem of *Bryophyllum pinnatum* and the root of *Phyllanthus amarus*.

Many of these differences suggest that they have varying nutrient compositions based on the species and the part of the plant matter utilized. These variations are crucial to comprehend to ensure proper use of these plants in ethnopharmacology and feeding regimes. The high levels of carbohydrates imply they could be energy sources (Okonwuet al., 2020), and the fluctuating moisture, fibre, and ash imply purposeful uses were present in various parts of the plants incorporated in herbal remedies. The analysis of the macro and micro elements of the minerals showed varying concentrations along the plant parts and species. In their analysis of the nutrient composition of Bryophyllum pinnatum, the authors noted higher potassium levels ranging from 189. 08 to 190. 35 mg/100g, which is important for enzyme activity as well as osmoregulation, according to White and Karley (2010). Another mineral that received more attention was phosphorus; the Bryophyllum pinnatum had a range of 29. 04 to 30. 03 mg/100 g phosphorus; phosphorus plays important roles in energy transfers as well as the synthesis of genetic materials (Raghothama, 1999).

However, the identified herbs, including a plant called *Phyllanthus amarus*, were found to have richer magnesium content ranging from 67. 89 to 69. 01 mg/ 100 g of the herb, which is a crucial nutrient for photosynthesis according to (Shaul, 2002). As with the findings of Okiki *et al.* (2015), the presence of these minerals is strongly affirmed because magnesium, phosphorus, and potassium were also found to be present in the leaves of *Phyllanthus amarus*.

Iron content is also higher in Phyllanthus amarus and supports its use in the traditional management of anaemia, as highlighted by Baye et al. (2017). Another cation found in significant levels was zinc; this is essential for immune function and protein synthesis (Prasad, 2013). Although it is not commonly discussed regarding plant requirements, sodium is involved in several physiological aspects. Plants need sodium in small quantities; however, they perform some functions of potassium and are involved in osmoregulation and maintenance of cellular turgidity (Bayeet al., 2017). Thus, given that Bryophyllum pinnatum and Phyllanthus amarus contain sodium, it gives credence to their utilization in traditional medicine to manage the electrolyte balance in the body. These phytochemical components are in agreement with the ethnopharmacological application of Bryophyllum pinnatum and Phyllanthus amarus. For instance, both plants contain alkaloids, which exhibit various pharmacological activities such as antimalarial, and anti-hypertensive analgesic, effects (Hosseinzadeh et al., 2015).

The tannins, which have an astringent effect, help in the healing of diarrhoea and wounds, among other uses (Salmerón-Manzano *et al.*, 2020). Saponins, working like a detergent, can be useful in reducing cholesterol and enhancing immunity (Naaz *et al.*, 2007). For instance, flavonoids have antioxidant properties that help in combating free radicals and prevent cellular damage and diseases such as chronic illnesses (Rani *et al.*, 2021). The identification of terpenoids adds to the medical uses of the plants due to their anti-inflammatory and anticancer activities (Patel *et al.*, 2011).

From the comparison of phytochemicals present in *Bryophyllum pinnatum* and *Phyllanthus amarus*, it is evident that *Bryophyllum pinnatum* contains increased phytochemical constituents and varieties, hence expanded utility in the treatment of diseases. The dissimilar distribution of flavonoids in *Bryophyllum pinnatum* roots and *Phyllanthus amarus* perhaps draws attention to the fact that certain parts of the plant are

more appropriate for varied applications in traditional medicine. Such specificity can improve the effectiveness of traditional medicines and reduce their side effects. The findings of this study provide direction in exploring the medicinal and pharmacological values of these plants. Further studies in the pharmacokinetic and bioavailability characteristics of the established phytochemicals would reveal their mode of action. Moreover, it would be important to investigate the synergistic effects of these compounds, which could lead to new discoveries of more effective medicines (Verpoorte, 2000). Additionally, the results of the mineral content show the dietetic importance of these plants, which indicates their applicability not only as medicine but also as a dietary supplement. The high place occupied by such obligatory microelements as potassium, magnesium, and phosphorus points to the existence of their therapeutic properties that can help manage a deficiency and maintain well-being (Gupta & Vaghela, 2019).

CONCLUSION

The proximate and phytochemical and minerals constituents showed that the compounds were present unevenly in various plant organs, with the leaf and stem exhibiting higher concentrations compared to the root. An understanding of these distribution patterns is essential in the selective use of different parts of these medicinal plants. Specifically, the leaves and stems, which have the highest concentrations of these beneficial compounds, maybe the most effective parts for therapeutic use. Also, knowledge of the distribution of these compounds and elements within plant organs can help in the strategic application of these plants in traditional medicine and diets for optimum health benefits.

REFERENCES

- Adeneye, A., & Benebo, A. (2008). Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus*on gentamicin and acetaminophen-induced nephrotoxic rats. Journal of ethnopharmacology, 118 2, 318-23. <u>https://doi.org/10.1016/j.jep.2008.04.025</u>.
- Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bulletin of the National Research Centre, 46(1), 87.
- Ameen, O.A., Hamid, A.A., Yusuf, Q., Njoku, O.G., Oseni, T.O., and Jamiu, W. (2021). Quantitative and Qualitative Assessment of Phytochemicals in Methanolic Extracts of Hurricane Weed (*Phyllanthus amarus*Schumach. &Thonn) Plant. J. Appl. Sci. Environ. 25 (2) 159–165.
- Araújo, E., Xavier-Santos, J., Silva, V., Lima, J., Schlamb, J., Fernandes-Pedrosa, M., Júnior, A., Júnior, R., Rathinasabapathy, T., Moncada, M., Esposito, D., Guerra, G., &Zucolotto, S. (2023). Gel formulated with *Bryophyllumpinnatum*leaf extract promotes skin wound healing in vivo by increasing VEGF expression: A novel potential active ingredient for pharmaceuticals. Frontiers in Pharmacology, 13. <u>https://doi.org/10.3389/fphar.2022.1104705</u>.
- Baye, K., Guyot, J. P., Icard-Vernière, C., & Mouquet-Rivier, C. (2017). Nutrient Intakes from Complementary Foods Consumed by Young Children in a Peri-Urban Village in Burkina Faso. *Nutrition Journal*, 16(1), 80.

- Chibli, L., Rodrigues, K., Gasparetto, C., Pinto, N., Fabri, R., Scio, E., Alves, M., Del-Vechio-Vieira, G., & Sousa, O. (2014). Anti-inflammatory effects of *Bryophyllumpinnatum*(Lam.) Oken ethanol extract in acute and chronic cutaneous inflammation. Journal of ethnopharmacology, 154 2, 330-8. <u>https://doi.org/10.1016/j.jep.2014.03.035</u>.
- Chinchansure, A. A., Anugamini, A., L., A., & Patel, R. (2024). Identification of authentic *Phyllanthus amarus*species in India. *Natural product research*, pp. 1–5. Advance online publication. https://doi.org/10.1080/14786419.2024.2313190
- Chopade, A. R., Pol, R. P., Patil, P. A., Dharanguttikar, V. R., Naikwade, N. S., Dias, R. J., & Mali, S. N. (2021). An Insight Into the Anxiolytic Effects of Lignans (Phyllanthin and Hypophyllanthin) and Tannin (Corilagin) Rich Extracts of *Phyllanthus amarus*: An *In-Silico* and *In-vivo* Approaches. *Combinatorial chemistry* & high throughput screening, 24(3), 415–422. https://doi.org/10.2174/1386207323666200605150915
- Fürer, K., Simões-Wüst, A., Mandach, U., Hamburger, M., &Potterat, O. (2016). *Bryophyllumpinnatum* and Related Species Used in Anthroposophic Medicine: Constituents, Pharmacological Activities, and Clinical Efficacy. Planta Medica, 82, 930-941. <u>https://doi.org/10.1055/s-0042-106727</u>.
- Gupta, M., & Vaghela, J. (2019). Recent Advances in Pharmacological and Phytochemistry Studies on Phyllanthus amarus. Pharmaceutical and Biosciences Journal. https://doi.org/10.20510/UKJPB/7/I1/179295.
- Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. *International Journal of Clinical Medicine*, 6(9), 635-642.
- Kamboj, A. &Saluja, A.K. (2009). Bryophyllumpinnatum (Lam.) Kurz.: Phytochemical and Pharmacological Profile: A Review. Phcog Rev., 3(6), 364–374.
- Mabeku, L., Bille, B., Tchouangueu, T., Nguepi, E., &Leundji, H. (2016). Treatment of Helicobacter pylori infected mice with Bryophyllumpinnatum, a medicinal plant with antioxidant and antimicrobial properties, reduces bacterial load. Pharmaceutical Biology, 55, 603-610. <u>https://doi.org/10.1080/13880209.2016.1266668</u>.
- Naaz, F., Javed, S., &Abdin, M. (2007). Hepatoprotective effect of ethanolic extract of *Phyllanthus amarus*Schum. Et Thonn. on aflatoxin B1-induced liver damage in mice. Journal of ethnopharmacology, p. 113 3, 503–9. <u>https://doi.org/10.1016/J.JEP.2007.07.017</u>.
- Ojewole, J. (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllumpinnatum*(Crassulaceae) leaf aqueous extract. Journal of ethnopharmacology, p. 99 1, 13–9. <u>https://doi.org/10.1016/J.JEP.2005.01.025</u>.
- Ogidi, O.I., Esie, N.G., and Dike, O.G. (2019). Phytochemical, proximate and mineral compositions of *Bryophyllumpinnatum*(Never die) medicinal plant. Journal of Pharmacognosy and Phytochemistry, 8(1), 629-6.

- Okiki, A. P., Olatunji, P. B., Egbebi, A. A. and Ojo, C. (2015). A Comparative Study of Nutritional and Phytochemical Composition of *Phyllanthus amarus*Leaf and Seed. American-Eurasian Journal of Toxicological Sciences, 7(4), 321-327.
- Okonwu, K., Ikechi-Nwogu, C. G. and Ivanhoe, M. (2020). Proximate Analysis, Phytochemical Properties And Antifungal Activity Of 'Miracle Plant', *Bryophyllumpinnatum*(Lam.) Oken. Nigerian Journal of Botany, 33(2).
- Owoyale, A. D. M., Galadimma, M., Daniyan, S. Y. & Adabara, N. (2019). Quantitative Phytochemical Analysis and Antifungal Susceptibility of Azardirachta indica against Some Strains of Candida albicans. Journal of Advances in Medical and Pharmaceutical Sciences, 21(4): 1-14.
- Patel, J., Tripathi, P., Sharma, V., Chauhan, N., & Dixit, V. (2011). *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. Journal of ethnopharmacology, 138(2), 286 – 313.
- Raghothama, K. G. (1999). Phosphate Acquisition. Annual Review of Plant Physiology and Plant Molecular Biology, pp. 50, 665–693.
- Rani, N., Lam, K., Jalil, J., Mohamad, H., Ali, M., & Husain, K. (2021). Mechanistic Studies of the Antiallergic Activity of *Phyllanthus amarus*Schum. &Thonn. and Its Compounds. Molecules, 26. <u>https://doi.org/10.3390/molecules26030695</u>.
- Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide Research Trends on Medicinal Plants. International journal of environmental research and public health, 17(10), 3376. <u>https://doi.org/10.3390/ijerph17103376</u>.
- Shaul, O. (2002). Magnesium Transport and Function in Plants: The Tip of the Iceberg. *BioMetals*, *pp. 15*, 309-323.
- Thorat, S., Shah, R., Mohite, S., & Patel, N. (2018). A review on *Bryophyllumpinnatum*. International research journal of pharmacy, 8, 1-3. <u>https://doi.org/10.7897/2230-8407.0812243.</u>
- Verpoorte, R. (2000). Pharmacognosy in the new millennium: leadfinding and biotechnology. J Pharm Pharmacol 52, 253–262.
- White, P. J., & Karley, A. J. (2010). Potassium. In P. Marschner (Ed.), Marschner's Mineral Nutrition of Higher Plants (3rd ed., pp. 351-382). Academic Press.
- Yao, A., Rasul, Z., Najmanová, I., Kamagaté, M., Said, A., Chabert, P., Auger, C., Die-Kakou, H., &Schini-Kerth, V. (2017). Beneficial effect of *Phyllanthus amarus* (Euphorbiaceae) on DOCA-salt-induced left ventricle cardiac hypertrophy and endothelial dysfunction in rats: O10. Biochemical Pharmacology, 139, 112–113. https://doi.org/10.1016/j.bcp.2017.06.075.