



## Research Article

# Comparative Phytochemical Constituents of Extracts of *Bryophyllum pinnatum* Grown in Anambra State, Nigeria

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### Abstract

*Bryophyllum pinnatum* is a plant abundant in several phytochemicals, that can be extracted, purified, and packaged for the goal of promoting public health. In this study, the phytochemical composition of the leaf extracts of *Bryophyllum pinnatum* grown in Anambra State, Nigeria, was compared. *B. pinnatum* leaves were obtained from a farm in Anambra State, Nigeria. The leaves were processed and extracted using cold water and hot water extraction, methanol extraction, and ethanol extraction. The extraction processes were carried out following standard extraction methods. Preliminary qualitative and quantitative analyses were conducted to determine the presence and concentration of phytochemicals in the plants. Phytochemicals tested include alkaloids, flavonoids, saponins, tannins, anthocyanins, reducing sugars, carbohydrates, proteins, cardiac glycosides, terpenoids, and phlobatannins. All the tested phytochemicals were recovered in the solvent (methanol and ethanol) extracts; carbohydrates, reducing sugar, protein, and anthocyanin were absent in the aqueous (cold and hot water) extracts. Varying concentrations of the phytochemicals in the different extracts were recorded, and they range from 0.00±0.00% to 13.30±0.22%, with the highest concentration of the extracts recovered in the solvent (methanol and ethanol) extracts. This study revealed that the extraction method used can influence the type and concentration of phytochemicals recovered in *Bryophyllum pinnatum*. Therefore, the specific desired phytochemical should determine the extract method to use in any study.

**Keywords:** *Bryophyllum pinnatum*; extraction; methanol; ethanol; aqueous

## Introduction

Native to Madagascar and southern Africa, *Bryophyllum pinnatum* is an environmental plant belonging to the Crassulaceae family. It thrives primarily in tropical climates, but it has also naturalized in several other places, such as the temperate regions of Asia, Australia, and New Zealand (Nagaratna, 2015). This succulent, glabrous herb grows

between 0.3 and 1.2 meters high. It is a common houseplant and a widely dispersed perennial medicinal herb. According to Ghasi et al. (2011), *B. pinnatum* also goes by the names Mother of Thousands, Air Plant, Maternity Plant, Love Plant, Miracle Leaf, Life Plant, and Lao di Sheng Gen.

In Nigeria, the plant is referred to as "African Never Die" locally, and folk medicine greatly values it. In tropical

America, India, China, Australia, and Africa, it has been used to treat a wide range of ailments, such as rheumatism, bodily discomfort, arthritis, heartburn, skin ulcers, peptic ulcers, diabetes mellitus, microbiological infections, and hypertension (Awoyemi *et al.*, 2012). The plant is especially well-known in Nigeria for its ability to effectively heal wounds and separate an infant's umbilicus. According to Yadav *et al.* (2016), *B. pinnatum* has been linked to some biological activities that may validate the plant's traditional uses. These activities include those that are immunomodulatory, CNS depressant, analgesic, anti-inflammatory, antimicrobial, antitumor, antiulcer, insecticidal, antidiabetic, anticonvulsant, antioxidant, and antihypertensive (Fernandes *et al.*, 2019). Niacin, riboflavin, thiamine, and ascorbic acid are all present in good amounts in the plant. For the body to function properly and for intercellular substances like collagen, bone matrix, and tooth dentine to grow normally throughout the body, natural ascorbic acid is essential (Akinyemi *et al.*, 2005); hence, the plant contributes to the proper functioning of the body system. Consequently, the plant is utilized in herbal therapy to cure ailments such as prostate cancer and the common cold (Mahata *et al.*, 2012).

Numerous active phytochemicals, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids, and organic acids, have been the subject of several studies, for example, Hua *et al.* (2022), Namadina *et al.* (2020), and Kouitcheu-Mabeku *et al.* (2017). The various pharmacological actions of the plant have been attributed to these substances. The extracts of *B. pinnatum* leaves, obtained either by cooking in water, pressing, roasting, or immersing in cold water for an entire night, can be used to cure a variety of ailments, including fevers, headaches, arthritis, joint and body pains, tonsillitis, diarrhea, and cough (Oliver-Bever, 1983). As several methods of extraction can be used for recovering the phytochemicals of the plants, the possibility of variation in the type and concentration of the constituents being impacted exists. Records show that aqueous extraction results in a higher concentration of polar compounds, while solvent extraction results in a higher concentration of non-polar compounds (Ahmad *et al.*, 2017).

The usage of herbal remedies like *B. pinnatum* has become common due to the high expense of conventional pharmaceuticals and the emergence of resistance to most conventional chemotherapy medicines, particularly in developing nations (Saad *et al.*, 2006). As the incidence of antibiotic resistance has increased and become a public concern, newer and safer antibiotics need to be introduced, and some plants are effective in this regard.

This study set out to compare the phytochemical composition of the leaf extracts of *Bryophyllum pinnatum*.

## Materials and Methods

### Study Area

The study was carried out in Anambra State, located in the southeast region of Nigeria. The state has 4,416 square miles. Anambra had a total population of 4,177,828 in 2006, with 2,117,984 males and 2,059,844 females. Most of the population was made up of farmers, traders, and civil servants. The climate of Anambra is tropical, humid, and dry. The annual temperature of the city is 84.180 °F (28.990 °C). Anambra has 243.38 wet days (66.68% of the total number of days) and averages 212.36 millimeters of precipitation a year.

### Collection of Plant Materials

The leaves of *B. pinnatum* used in this study were obtained from a backyard farm located at Omagba, Anambra State, Nigeria (Fig. 1).



Fig. 1: *Bryophyllum pinnatum* bred at Anambra State, Nigeria

### Sample Processing

The plant's leaves were cleaned under running water and allowed to air dry for two weeks at room temperature in the shade. The dried leaves were ground into a coarse powder using a sterile electric blender that had been cleaned with 100% alcohol. The powder was then kept at room temperature in an airtight bottle (Wrona *et al.*, 2019).

### Extract Preparation

Aqueous extract was made using hot water, and 10 g of the plant's fine powder was added to a 100-ml flask along with 20 ml of distilled water. This was brought to a boil using a hot plate for an hour, and then it was filtered through Whatman No. 1 filter paper for three minutes to recover a filtrate (Karabi, 2015).

The solvent extracts were made using the technique outlined by Karabi (2015). It was made using a 1:2 ratio (10 g of the plant's powder combined with 20 ml of methanol and ethanol separately). For four hours, the mixture was let stand at room temperature, stirring now and then. To get rid of the leftovers, the mixture was filtered separately through Whatman No. 1 filter paper. The ethanol and methanol content in the filtrate was allowed to evaporate at room temperature for two days after it was allowed to settle for thirty minutes.

Before being used, the concentrated aqueous, ethanol, and methanol extracts were kept in a refrigerator at 5°C.

### **Qualitative Evaluation of the Plant's Phytochemical Constituents**

In this study, alkaloids, tannins, saponins, reducing sugar, anthocyanin, flavonoids, carbohydrates, protein, terpenoids, cardiac glycosides, and phlobatannins were found using the standard method for figuring out phytochemicals (Mann *et al.*, 2008).

#### *Test for Alkaloids*

Three millilitres (3 ml) of the extract were tested for alkaloids using Dragendorff's reagent. The reagent was added to the extract and allowed to sit for 5 minutes. The formation of an orange-brown precipitate revealed the presence of alkaloids (Mann *et al.*, 2008).

#### *Test for Tannins*

To three millilitres of the extract, a ten per cent (10%) alcoholic ferric chloride solution was added. The formation of dark blue coloring revealed the presence of tannins (Mann *et al.*, 2008).

#### *Test for Saponins*

The plant extract was mixed with three drops of olive oil and given a good shake. The formation of a soluble emulsion suggested the presence of saponin (Mann *et al.*, 2008).

#### *Test for Reducing Sugar*

In a test tube, Fehling's solutions (A and B) were combined with the plant extract at a ratio of 1:1. Color changes from green to light green indicated the presence of reducing sugar (Mann *et al.*, 2008).

#### *Test for Anthocyanin*

The plant extract (3 ml) was combined with a few drops of 10% sodium hydroxide. Precipitation with a dark blue coloration suggested the existence of this phytochemical (Mann *et al.*, 2008).

#### *Test for Flavonoids*

A small amount of sodium hydroxide solution was applied to 3 ml of the extract and allowed to sit for 2 minutes. When 1% HCl was added, a color shift from yellow to colorless was seen, which suggested the presence of flavonoids (Mann *et al.*, 2008).

#### *Test for Carbohydrates*

The carbohydrate content was determined using Benedict's reagent. Three millilitres of the extract were mixed with Benedict's reagent and heated in a water bath. The formation of an orange-red precipitate indicated the presence of carbohydrates (Mann *et al.*, 2008).

#### *Test for Proteins*

A 1 ml 40% NaOH, 2 ml cupric sulfate, and 2 ml plant extract mixture was obtained. The purple precipitation indicated the presence of protein (Mann *et al.*, 2008).

#### *Test for Terpenoids*

The plant extract (3 ml) was supplemented with thionyl chloride. The emergence of a pink colour indicated the presence of terpenoids (Mann *et al.*, 2008).

#### *Test for Cardiac Glycosides*

The plant extracts were supplemented with concentrated sulfuric acid, ferric chloride, and glacial acetic acid. The mixture's green color revealed the presence of cardiac glycosides (Mann *et al.*, 2008).

#### *Test for Phlobatanin*

A few drops of 2% hydrochloric acid were added to the plant extract and heated to a boil. The presence of light-crimson precipitation determined phlobatannins (Mann *et al.*, 2008).

### **Quantitative Phytochemical Analysis of the Plant Extract**

#### *Estimation of Alkaloids*

The alkaloid content was determined using the description provided by Nwaka *et al.* (2018). After measuring a mass of five grams (5g) of the sample and placing it in a 250-ml beaker, 200 ml of a solution containing 20% acetic acid in ethanol was added. The beaker was then sealed, and the mixture was allowed to sit at a temperature of 250 °C for 4 hours. Filter paper No. 42 was employed for the filtration process, and the resulting liquid was condensed to 25% of its initial volume using a water bath (Memmert). The extract was gradually treated with concentrated ammonium hydroxide to induce the formation of a precipitate. After allowing the mixture to fully settle, the solid that formed was separated, washed with a diluted solution of NH<sub>4</sub>OH (1% ammonia), and filtered using pre-weighed filter paper. The alkaloid residue on the filter paper was subjected to drying at a temperature of 800 °C using a precision electrothermal type BNP 9052 England oven. The residue was then weighed after the drying process. The percentage of the sample weight containing alkaloids was calculated using the following formula:

$$\% \text{ weight of alkaloid} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times 100$$

#### *Estimation of Flavonoids*

The method for estimation of flavonoids used in this study adhered to the description provided by Odeyemi *et al.* (2023). Under normal conditions, a quantity of ten grams (10g) of the plant sample was repeatedly extracted using one hundred millilitres of an aqueous methanol solution with an 80% concentration. The entire mixture was filtered using a pre-weighed Whatman No. 42 filter paper. Subsequently, the filtrate was transferred to a crucible, subjected to desiccation using a water bath, and then measured to verify its consistent weight.

$$\% \text{ weight of flavonoid} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times 100$$

### Estimation of Saponins

The protocols described by Obadoni and Ochuko (2001) were adhered to determine the Saponin concentration. Following the addition of a five-gram (5g) sample to a solution of 20% acetic acid in ethanol, the mixture was allowed to sit undisturbed for a duration of 24 hours at a temperature of 500 °C. The extract underwent filtration, followed by concentration using a water bath to reduce its volume to one-fourth of the original amount. The extract was gradually treated with concentrated NH<sub>4</sub>OH, added in small increments, until the precipitate was completely produced. Following the complete settling of the mixture, the precipitate was separated using filtration and its weight was determined. The saponin content was quantified by calculating a percentage using a specific formula.

$$\% \text{ weight of saponin} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times 100$$

### Estimation of Cardiac Glycosides

The methodology proposed by Wang *et al.* (2017) was implemented. Two distinct solutions were introduced into a volume of one milliliter of extract: one containing 2% 3,5-DNS (dinitro salicylic acid) dissolved in methanol, and the other containing 5% NaOH dissolved in water. Following a two-minute boiling period, a brick-red solid formed, prompting the filtration of the boiled sample. The weight of the filter paper was measured before it was filtered. The filter paper, containing residue, was dried in an oven at a temperature of 500 °C until it reached a state of total dryness. The weight of the dried filter paper was then measured and recorded. The cardiac glycoside concentration was quantified using a mathematical technique.

$$\% \text{ weight of cardiac glycosides} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times$$

100

### Estimation of Tannins

The method described by Wang *et al.* (2017) which presented a detailed elucidation of the Follinsdennis titration approach was used to determine the tannin concentration. A volume of 100 ml of petroleum ether was introduced into a conical flask containing 20 g of the extract. The mixture was then hermetically sealed for a duration of 24 hours. Following the filtration of the mixture, the sample was left undisturbed for a duration of 15 minutes to facilitate the evaporation of the petroleum ether. Subsequently, it was extracted again by a 4-hour soaking process in a solution consisting of 100 milliliters of ethanol with 10% acetic acid. Subsequently, the sample underwent filtration using pre-weighed filter paper, followed by drying in an oven and subsequent weighing. The tannin's weight percentage was determined using the following formula:

$$\% \text{ weight of Tanin} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times 100$$

### Estimation of Anthocyanin

This was carried out with the gravimetric approach as outlined by Harborne (1998). For thirty minutes, five grams (5g) of the extract were boiled in 100 milliliters of 2 M HCl. Whatman filter paper was utilized to filter the hydrolysate. After pouring the filtrate into a separation funnel, the same volume of ethyl acetate was added, stirred, and given time to separate into two layers. The aqueous layer was disposed of, and the ethyl-acetate layer was recovered. The anthocyanin was subsequently extracted from the dry extract using 50 ml of strong amyl alcohol. The alcohol extract was dried after filtering with pre-weighed filter paper. The anthocyanin weight was calculated and reported as a percentage of the original sample.

$$\% \text{ weight of anthocyanin} = \frac{\text{Weight of filter paper with filtrate} - \text{initial weight of filter paper}}{\text{weight of sample analyzed}} \times 100$$

### Estimation of Crude Protein

Harborne (1998) described how to use the Micro-Kjeldahl Method. About 2 grams of the sample were taken in a Kjeldahl flask, and 10 grams of sodium sulfate and 0.5 grams of copper sulfate were added and mixed well. A few glass beads were added to the flask to prevent spurting while heating. Then 25 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added and heated for 15-20 minutes in an inclined position. The solution was boiled until a greenish color was obtained. It was allowed to cool. About 100 ml of distilled water was added to the Kjeldahl flask, shaken properly, and transferred into a 250 ml volumetric flask. Then the final volume was made up to 250 ml by adding distilled water. In a conical flask, 10–15 ml of 2% Boric acid were taken, and the flask was placed below the condenser of the distillation apparatus. Thereafter, 5 ml of aliquot was transferred to the Micro Kjeldahl steam distillation apparatus, along with 1 drop of phenolphthalene and 10–15 ml of 40% NaOH. The distillation was carried out for 5–10 minutes until ammonia was free from the aliquot. The distillation product was then titrated against N/10 H<sub>2</sub>SO<sub>4</sub>.

$$\% \text{ of Nitrogen} = \frac{\text{ml of } \frac{N}{10} \text{H}_2\text{SO}_4 \text{ used up} \times 250 \times 0.004 \times 100}{\text{Volume of aliquot} \times \text{gm of the substance taken}}$$

$$\% \text{ of crude protein} = \% \text{ Nitrogen} \times 6.25$$

### Data Analysis

Each analysis was conducted in triplicate. The average of the values obtained was calculated, and the results were recorded as the average ± standard deviation.

### Results and Discussion

The analysis of *Bryophillum pinnatum* extracts provides important information about the phytochemical profile of the plant. This information can be used to identify potential medicinal or therapeutic compounds and to understand the plant's chemical makeup. Additionally, analysis of the

extract can be used to identify and quantify the active ingredients, which can be useful for the standardization and quality control of herbal products (Balekundri and Mannur, 2020). In this study, the qualitative and quantitative analyses of *B. pinnatum* were determined comparatively for the various extraction methods: cold water, hot water, ethanol, and methanol extraction.

In this study, Table 1 shows the qualitative analysis of the different extracts of *B. pinnatum*. The constituents that have been identified in cold water extracts include flavonoids, alkaloids, cardiac glycosides, tannins, and saponins. However, additional constituents, such as phlobatannins and terpenoids, were recovered in hot water extract in hot water extract. In methanol and ethanol extraction, the constituents identified were alkaloids, tannins, cardiac glycosides, flavonoids, terpenoids, proteins, carbohydrates, saponins, reducing sugars, phlobatannins, and anthocyanins. The qualitative analysis of *B. pinnatum* presents the difference in constituents of extracts due to the difference in the extraction process used. In comparison to aqueous extraction, solvent extraction recovered a greater number of phytochemicals (Table 1). This may be attributed to the impact of the polar nature of the solvents used on the plant matrices; methanol and ethanol were effective at permeating the matrix of the plant leaves and driving out the components of the leaf.

The result of this study corroborates the reports of several studies. Yadav *et al.* (2016) revealed the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids, and steroids in alcohol extracts of *B. pinnatum* during their study on the application of *Bryophyllum pinnatum* leaf extracts in lithiatic rats against the formation of renal calculi. Hot water extraction by Ogidi *et al.* (2019) revealed the presence of alkaloids, tannins, saponin, terpenoid, glycoside, phenols, and flavonoids. Nguelefack (2006) reported that alkaloids and saponins are present in the aqueous and alcoholic extracts of the leaves of *B. pinnatum*. These phytochemicals contained in *B. pinnatum* are responsible for the antibacterial and other medicinal properties possessed by the plant (Mahata *et al.*, 2012).

The quantitative phytochemical analysis of the plant extract is presented in Table 2. The extract constituents in 10g of the plant leaf ranged from 0.00±0.00% to 13.30±0.22%. The aqueous extracts had the most alkaloids (6.65±0.02% for the cold-water extract and 8.20±0.05% for the hot water extract). The solvent extracts had the most proteins (9.50±0.18% for the methanol extract and 13.30±0.22% for the ethanol extract). The result shows that a higher concentration of the phytochemicals was recovered using the solvent extraction process.

**Table 1:** Qualitative analysis of *Bryophyllum pinnatum* leaf

Phytochemical	Composition			
	Cold water	Hot water	Methanol	Ethanol
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Terpenoids	-	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Proteins	-	-	+	+
Carbohydrate	-	-	+	+
Saponin	+	+	+	+
Reducing sugar	-	-	+	+
Phlobatannins	-	+	+	+
Anthocyanin	-	-	+	+

**Table 2:** The quantitative analysis of some phytochemical components of *Bryophyllum pinnatum*.

Phytochemical	Percentage weight (%)			
	Cold water	Hot water	Methanol	Ethanol
Alkaloids	6.65 ± 0.02	8.20± 0.05	7.94± 0.01	7.45± 0.02
Flavonoids	2.26± 0.01	3.55± 0.01	8.54± 0.02	10.91± 0.12
Saponins	1.24± 0.01	1.30± 0.02	3.34± 0.02	4.95± 0.02
Tannins	2.88± 0.10	4.82± 0.00	3.89± 0.12	3.03± 0.01
Anthocyanins	0.0± 0.00	0.16± 0.01	3.24± 0.02	3.26± 0.01
Cardiac glycosides	1.67± 0.02	3.8± 0.10	4.44± 0.02	5.22± 0.01
Proteins	0.11± 0.00	0.06± 0.02	9.50± 0.18	13.30± 0.22

Various studies show different concentrations of the phytochemicals in *B. pinnatum* leaf extracts. The researchers, Ujah and Onyishi (2023) found that terpenoid was the most abundant phytochemical in *B. pinnatum* that was extracted with water. In a different study by Imaobong et al. (2020), polyphenols were found to be the most abundant, with  $34.49 \pm 0.47$  and  $21.2 \pm 2.2$  for the methanol and ethanol extracts, respectively. The variations in phytochemical concentration recorded in our study and others can be attributed to the method of testing used for the various phytochemicals tested.

## Conclusion

In this study, several phytochemicals were recovered that are of medical and pharmaceutical importance. Solvent (methanol, ethanol) extraction and aqueous extraction (cold water, hot water) are distinct methods used for extracting substances from raw materials. The different methods tested in this study recovered varying types and concentrations of phytochemicals.

However, the choice between the extraction methods depends on the specific characteristics of the desired extraction process and the properties of the substances involved. More than one extraction method is recommended during studies on *B. pinnatum* to ensure all bioactive components of the plant are recovered. Further studies are required on the extraction, purification, identification, and structure elucidation of the phytochemical constituents of the plants.

## Authors' Contribution

Nnaebue ND, Soludo Obumneme C. & Onuorah Samuel C designed the research plan; Anyaoha Victoria I. & Ajogwu Tobeckukwu MC performed experimental works & collected the required data. Soludo Obumneme C., Isiaka Amarachukwu B & Onuorah Samuel C analysed the data; Anaukwu CG prepared the manuscript, critical revised and finalized the manuscript. Final form of manuscript was approved by all authors.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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