DETERMINATION OF THE BIOCHEMICAL (PHYTOCHEMICALS, PROXIMATE AND MINERAL) COMPOSITIONS OF Bryophyllum pinnatum LEAF (MIRACLE LEAF)

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Abstract

This work highlights on assessment of the biochemical composition of *B. pinnatum* leaf. The phytochemical analysis were performed by standard analytical method using ethanol as solvent. The phytochemicals found in the extract are alkaloids (++), flavonoids (+++), saponins (+++), tannins (+++), phenols (++), terpenoids (+) and glycosides (+). The proximate analysis was done using standard methods of analysis as described by AOAC. The results from the investigation indicated that moisture (74.51%), ash (0.80%), fat (1.15%), fibre (6.90%), protein (4.30%) and carbohydrate (12.34%). Analysis of mineral contents of *B. pinnatum* was done using titrimetric method. The results of the analysis indicated that *B. pinnatum* had calcium (38.47ppm), Magnesium (18.53ppm), potassium (17.17ppm) and iron (2.21ppm). These inherent potentials in *B. pinnatum* could be the reason the plant is recommended for the treatment of various ailments.

KEYWORDS: *Bryophyllum pinnatum*, Phytochemicals, Proximate, Minerals, Ailments, Ethanolic.

INTRODUCTION

The World Health Organization (WHO) defines medicinal plants as those with one or more of parts contain compounds that can be utilized therapeutically or as a starting point for the production of effective medications. However, it has been reported that medicinal plants are widely employed for the treatment of a number of ailments (Grover, *et al*., 2022). To avoid mistaken destruction of plants, which could serve as a spring board for the development of potent medications, it is necessary to examine their various therapeutic potentials. According

to numerous studies on medicinal plants and herbal drug synthesis the bioactive components of medicinal plant found in the leaves, roots, stem and other plant parts. These bioactive compounds popularly referred to as phytochemicals, include terpenes, alkaloids, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraquinones (Iwu, 2023 and Imaobong, *et al.*, 2020). Many plants produce secondary metabolites with antioxidant, antibacterial, antifungal and other biological potentials (Mehrorosh, *et al.*, 2020). According to previous studies, approximately 80% of the world's population still relies on traditional medicines for their primary health care, the majority of which uses plant extracts (Sandhya, *et al.*, 2023). In the past few years, have seen a rise in the demand for natural substances like plant-rich antimicrobials due to concerns about the safety of synthetic antimicrobial medications (Imaobong, *et al.*, 2020). This is due to report that herbal medicines, especially when compared with synthetic medications, are believe to be side effects-free, accessible, inexpensive and safe (Britton, *et al.*, 2022 and Hussain, *et al.*, 2020). One of such plants used to treat variety of diseases brought on by pathogenic organisms is *Bryophyllum pinnatum. B. pinnatum* plants are in the Crassulaceae family and the common names include life plant, African never die, love plant, miracle leaf, and Canterbury bells, are also known "Shuka halinka or Karan massalaci" in Hausa, a northern Nigerian tribe (Mudi and Ibrahim, 2021). Tropical Africa, America, Hawaii, India, China, Australia, and Madagascar all have significant populations of it (Afzal, *et al.*, 2023). *B. pinnatum* is a succulent, 50 – 202 cm tall and about 3.2 cm wide plant that can reproduces vegetatively from leaf bulbils and through seed (Imaobong, *et al.*, 2020). To treat antiinflammatory, antipyretic, antimicrobial, antioxidant, antitumor, antidiabetic, antiulcer, antiseptic, hypocholesterolemic, and cough suppressant conditions, however, the leaves and leaf juice have been employed (Ali, *et al.*, 2021). Nonetheless the plant is rich in valuable compounds including polyphenols, tannins, glycosaponins, flavonoids, steroidal glycosides and many other crucial chemical components that are responsible for its anti-oxidant, anti-pyretic, anti-inflammatory, anti-arthritic, anti-allergic, analgesic, antiseptic, sedative, anti-depression, wound healing, hepatoprotective, nephroprotective, tocolysis, urolithic, anti-psychotic, muscle relaxant, anti-protozoal, anti-microbial and anti-diabetic properties. Moreover, the herb is good source of carbohydrates, proteins, amino acids, lipids, vitamins and mineral elements such as Na, Ca, K, P, Mg, Mn, Fe, Zn. The presence of zinc in the plants may indicate that it can be useful in managing diabetes, which is brought on by ineffective response to insulin. Despite the fact that many aspects of the herb have already explored, it is still required to conduct additional in-depth research of the

herb to prove it therapeutic value and evaluate the justification for its usage in traditional medicines.

Therefore, the current study aims to examine the biochemical compositions of *Bryophyllum pinnatum* leaf.

MATERIALS AND METHODS

Sample collection and preparation

The fresh leaves of *Bryoplyllum pinnatum* was collected from Mr Okoli's compound at Oko, Orumba North Local Government Area, Anambra State.

Materials and Reagent

Instrument and glasswares used for this work were collected from Science Laboratory Technology Department, Federal Polytechnic Oko, while Chemicals and reagents were of analytical grade and standard.

Methods

The collected plant material (*B. pinnatum* leaves) was air dried for 5 days and ground to powder. The ground sample was stored in an air tight container until needed for analysis.

Extraction of plant materials

50g of macerated *Bryoplyllum pinnatum* was extracted using 500ml of ethanol (ethanolic extraction). The extraction was done in 1000ml conical flask.

The conical flask was plugged with rubber cork; then be shaken at 120ml for 30 minutes at each interval for 4 hours and was allowed to stand at room temperature for 24hour at this juncture, the extracts was decanted, filtered using sterile white man no 1 filter paper. The extracts was concentrated and a crude extract was obtained. It was screened for secondary metabolites.

Qualitative Phytochemical Screening of the *Bryoplyllum pinnatum*

The crude extract of *Bryoplyllum pinnatum* was screened using the standard laboratory technique (Harborne, 2022).

Test for Alkaloids using Wagner's Test

2ml of the ethanolic extract and 5ml of wagner's reagent was added. A chocolate brown precipitate was observed.

Test for Tannins using Bromine Water Test.

2ml of ethanolic extract solution was added to bromine water in a test tube a light yellow coloured solution was observed.

Test for flavonoids Using Magnesium (II) Chloride Test

2ml of the plant extract was added to 4ml of magnesium(II) chloride solution in a test tube followed by 1ml conc H_2SO_4,. a pale yellow coloured solution was obtained.

Test for Saponin Using Frothing Test

5ml of ethanolic extract was poured into a test tube and 10ml of distilled water was added to it and the mixture was shaken vigorously for about 2 minutes. A persistent Frothing was observed.

Test for Terpenes/Steroids

2ml of Chloroform extract was placed in a test tube and two drops of acetic anhydride and 1ml of conc. H_2SO_4 was added. A brownish solution was observed.

Test for Terpenoids

One (1) millitre of the sample extract was mixed with 3ml of concentrated sulphuric acid, the colour at the formation of a ring beneath indicate the presence of terpenoids.

Test for Glycosides

In separate test tubes, 10 ml of 50% H_2SO_4 was added to 1 ml of the filtrate extract. The mixture was then heated for 15 minutes before 10 ml of the Fehling's solution was added and the mixture was boiled. A brick red precipitate was observed.

Proximate Analysis of *Bryoplyllum pinnatum*

Moisture Content Determination

The method described by AOAC, (2021) was adopted. The method is based upon the removal of water from the sample and its measurement by loss of weight.

A clean crucible was weighed and dried in the Oven (W_1): 1.0g of the sample was weighed into the crucible (W_2) and dried at 100^oC for some minutes the crucible was then transferred, from the oven to desiccators, cool and was reweighed (W_3).

The percentage (%) moisture content was calculated from:

$$\textbf{\% Moisture Content} = \frac{W_{2-}W_3}{W_{2-}W_1} \times \frac{100}{1}$$

Crude Protein Determination

Kjeldahl method was used (AOAC, 2021). This method was divided into three (3) namely: digestion, distillation and titration.

Digestion: Approximately 0.1g of ground air dried miracle leaf sample was weighed into clean dried Kjeldahl flask for digestion and 0.1g (Copper tetraoxosulphate (iv) crystal. 0.5g sodium teraoxosulphate (iv) crystal and 25ml of concentrated H_2SO_4 acid was added into the flask and some glass beads were added into the flask content as an anti-bumping agent. The Kjeldahl flask and its content was transferred to the digesting chamber in a fume cupboard and digested.

Distillation: Out of the homogenous solution of the digest. 20ml was transferred into a distillation flask using a pipette. Then 20ml of 40% sodium hydroxide solution was added carefully down the side of the flask through a funnel. After this, 50ml of 2% boric acid solution was pipetted into a receiving flask and two drops of methyl red indicator was added. The distillation unit was fitted such that the condenser was connected to the receiving flask through a funnel then 2% Boric acid solution was pipette reoccurring flask and two drops of methyl red indicator was added. The distillation unit was fitted such that the condenser was connected to the receiving flask with a glass tube and the condenser cooled with constant supply of cold water from tap. Also, the tip of the glass tube was immersed in the boric acid. The distillation unit was then heated on a heating mantle for about 35 minutes until the pink solution of the boric acid turned blue and the volume increased to about 100ml by the distillate.

Titration: Ten millilitres (10ml) of the distillate was titrated against 0.1N hydrochloric acid to a colourless end point. A blank solution was also titrated to get recorded; the percentage crude protein was calculated as follows.

% crude protein = % Nitrogen x 6.25

Where % Nitrogen = $\frac{28 \times ut - vb}{100 \quad W_o}$

V_t = titre volume of sample

V_b = titre volume of blank

W_o = weight of sample

Ash content Determination

The AOAC, (2021) method was used, the porcelain crucible was dried in an oven at 100^{o}C for 10mins, cooled in a desiccator and weighed (W_1). Two gram of the sample was placed into the previously weighed porcelain crucible and was reweighed (W_o) and the placed in the furnace for few hours at 600^{o}C to ensure proper ashing. The crucible containing the ash was removed cooled in the desiccator and weighed (W_3). Ash content was calculated as %.

$$\% \quad = \quad \text{Ash content} \quad = \quad \frac{W_3 - W_1}{} \quad x \quad \frac{100}{}$$

$$\frac{W_2-W_1}{1}$$

Fat Content Determination

The fat content was determined as in the AOAC (2021). A clean, dried 500ml round bottom flask containing few anti-bumping granules was weighed (W_1) and 150ml ethanol and normal –haxane was weighed transferred into the flask fitted with soxhlex extraction apparatus. Round bottom flask and a condenser were connected to the soxhlet extractor and cold water circulation was put on and the heating mantle was switched on and the heating was adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for an hour. The round bottom flask and extracted oil was cooled and the weighed (W_2). % crude fat content =

$$\frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of Crude Fibre

The method described by AOAC, (2021) was used 2.0g of the finely ground sample was weighed out into a round bottom flask. 10ml of 1.25% suphuric acid solution was added and the mixture boiled under a reflux for 30mins. The hot solution was quickly filtered under suction. The insoluble matter washed several times with hot water until it was acid free. It was carefully transferred into the flask and 10ml of hot 1.25% sodium hydroxide (NaOH) solution was added and the mixture boiled again under reflux for 30minutes and was quickly filtered under suction. The insoluble residue was dried to constant weight in the oven at a 105^{oC} cooled in a desiccator and weighed the sample furnance at $300^{o}C$ for about 30 mins cooled in the desicators and reweighed (C_2), the loss in weight of sample of incineration = $C_1 - C_2$

$$\textbf{\% crude fibre} = \frac{C_1 - C_2}{\text{Weight of original sample}} \times \frac{100}{1}$$

Determination of Carbohydrate

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude fat, crude protein, crude fibre was subtracted from 100. Total carbohydrate = 100 – (% moisture + % Ash+% fat + % protein + % fibre) (AOAC, 2021).

Mineral Analysis of *Bryoplyllum pinnatum*

The sample was weighed and subjected to drying in a well clean nickel crucible at $55^{o}C$ in a muffle furnace. The resultant ash was dissolved in 5.0ml of $HNO_3/HCL/H_2O$ (1:2:3) and heated gently on a heating muffle until brown fumes disappeared. 5.0ml of distilled water was added to the sample in crucible and heated until colourless solution was obtained. The mineral solution was filtered into a 100ml volumetric flask through filter paper, and the volume was

made to the mark with distilled water. The solution was analyzed in triplicate for its elemental composition using titrimetric method.

The minerals to be analyzed are;

Calcium

Magnesium

Iron and

Potassium

Quantitative Analysis of Calcium, Potassium, Magnesium and Iron.

The *Bryophyllum pinnatum* sample (5g) with concentrated nitric acid (20ml) was placed in a small dish and heated slowly to boil for 2 hours. After cooling, 10ml deionized water was carefully added and the mixture boiled for 3mins. After cooling, the sample was filtered with filter paper in volumentric flask (1000ml) and the volume made to 1 litre with de-ionized water. The pure calcium, potassium chloride, copper sulphate and iron (ii) chloride was incubated in oven ($110^{o}C$) to dehydrate for 1 hour and then transferred to a desiccator. The compounds were dissolved in 1000ml double distilled water to make a serial concentration of standards, 1ppm, 2ppm, 3ppm, 4ppm, 5ppm and 10ppm. The *Bryophyllum pinnatum* leaf was dissolved in HNO_3(10ml) and then diluted 10 times with distilled water.

Titrimetric method was used for determination of calcium, potassium, magnesium and iron by standard curves.

RESULTS

Table 1: Qualitative Phytochemical Analysis of *Bryophyllum pinnatum*

Parameters	**Intensity**
Alkaloids	++
Flavonoids	+++
Saponins	+++
Tannins	+++
Terperiods	+
Glycosides	+
Phenols	++

Key

+++	Highly present
++	Moderately present
+	Fairly Present

- Negative

Table 2: Proximate Analysis of *Bryophyllum pinnatum*

Parameters	Values (%)
Moisture	74.51
Ash	0.80
Fat	1.15
Fibre	6.90
Protein	4.30
Carbohydrate	12.34

Table 3: Mineral Contents of *Bryophyllum pinnatum*

Minerals	Concentration (ppm)
Calcium (Ca)	38.47
Magnesium (Mg)	18.53
Iron (Fe)	2.21
Potassium (K)	17.17

DISCUSSION

The results in this study revealed the presence of the following phytochemical constituents; alkaloids (++), flavonoids (+++), phenols (++), saponins (+++), tannins (+++), Terperiods (+) and glycosides (+) in the extract as presented in table 1.

The tannin content of *B. pinnatum* leaves was higher than the range reported by Uyoh *et al.* (2023) for *T. tetraptera* fruits. According to Chikezie *et al.* (2021), tannins are considered as dietary anti-nutrients responsible for the astringent taste and poor palatability of foods and drinks. The phenolics are known for their antiinflammatory, anticlotting and antioxidant properties and are also immune enhancers and hormone modulators. Flavonoids are known for their ability to enhance the effects of ascorbic acid along with vitamin C. The biological functions of flavonoids include actions against allergies, inflammation, microbes, ulcers, hepatotoxin viruses and tumors. Flavonoids in the intestinal tract are known to lower the risk of heart disease (Okwu, 2020). The alkaloid levels of *B. pinnatum* shows that the plant could be used as CMS stimulants and as a powerful pain reliever. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties

and bitterness. These properties indicate a high medicinal value of the plant. These may be the reason *B. pinnatum* is used in the treatment of wounds, burns and ulcer in herbal medicine. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes. This may explain why traditional medical practitioners in the South Eastern Nigeria use *B. pinnatum* in treating wounds and burns (Nwali *et al*., 2020).

The results of this study collaborate the reports of several studies. Yadav et al. (2016) revealed the presence of phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids, and steroids in ethanolic extracts of *B. pinnatum* during their study on the application of *Bryophyllum pinnatum* leaf extracts in lithiatic rats against the formation of renal calculi. ethanolic extraction by Ogidi et al. (2019) revealed the presence of alkaloids, tannins, saponin, terpenoid, glycoside, phenols, and flavonoids. Nguelefack (2023) reported that alkaloids and saponins are present in the ethanolic 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 obtained data for the proximate analysis of *B. pinnatum* indicated that the moisture content of *B. pinnatum* was 74.51%, it has high water content. It has low life span, the ash content was low having the value of 0.80% which means that it has enough minerals. The fat content was 1.15% which means it has low oil quantity, the fibre content was 6.90% which means that it has sugar content which is not good for diabetic patients, the protein content was 4.30% which means that it does not enhance growth and carbohydrate content was 12.34% which means it gives energy. The results provided additional information about the nutritional value and confirms that *B. pinnatum* is an interesting healthy food.

Table 3, presents the mineral constituents of *B. pinnatum*, calcium concentration was 38.47ppm, magnesium was 18.53ppm, potassium was 17.17ppm and iron was 2.21ppm were obtained from *B. pinnatum*. Normal extracellular calcium concentration is necessary for blood coagulation and for the integrity, intracellular substance. It also helps in the development of strong bone and teeth. Trease and Evans (2019) reported that high potassium content obtained in *B. pinnatum* plays a vital role in normal cell function including neuro-transmission, muscle contraction and for maintaining acid-base balance. According to Grosvernor and Smolin (2022), minerals in food are required for diverse metabolic functions. Low mineral concentrations in crops can be linked to the deficient nature of the soil where crops are found (Bouis, 2020; White and Broadley, 2021).

CONCLUSION

The study revealed that *B. pinnatum* contains appreciable levels of carbohydrates, proteins, fat, oil, crude fiber, ash, moisture and mineral contents, tannin, saponin, flavonoids, alkaloids, glycoside, terpeniods and phenolic compounds in the leaves. The leave of *Bryophyllum*

pinnatum contain phytochemicals, proximate and minerals in appreciable quantities and they possess activities like anti-diabetic, anti-ulcer, anthelmintic, immunosuppressive, hepatoprotective, anti-nociceptive, antiinflammatory, nephroprotective, antioxidant, analgesic, anticonvulsant, neuropharmacological, antipyretic, and antihypertensive. Bryophyllurn pinnatum is also used in the treatment and prevention of infections. As a rich source of secondary metabolites Bryophyllum pinnatum can be a potential source of useful drugs. Incorporation of this plant in diet as nutraceuticals is worth recommendation. Moreover, they are ubiquitous, can be grown or cultivated and is not even endangered (Odangowei *et al.*, 2019). This also shows *that B. pinnatum* is a good source of nutrition for humans and should be incorporated in our daily diet.

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