Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 629-635
Received: 07-11-2018
Accepted: 08-12-2018

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Phytochemical, proximate and mineral compositions of *Bryophyllum Pinnatum* (Never die) medicinal plant

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Abstract
Bryophyllum pinnatum is an indigenous and exotic plant used widely by the traditional practitioners for treating various ailments like renal calculi, hypertension, asthma, cold, abscesses, bleeding disorders. Phytochemical, proximate and mineral compositions of leaves, stems and roots of *Bryophyllum pinnatum* (Never die) plant was analyzed using the standard method of Association of Analytical Chemist (AOAC) and Atomic Absorption Spectrophotometeric (AAS) method. The qualitative phytochemical result showed that plant samples contains alkaloids, tannins, saponin, terpenoid, glycoside, phenols and flavonoid which was absent in the roots of the plant. Quantitative phytochemical results ranges from alkaloid 18.72 ± 5.70%, tannins 12.40 ± 3.64%, saponin 2.46 ± 1.20%, flavonoid 2.36 ± 0.98%, terpenoid 3.77 ± 2.88% and phenol 10.48 ± 8.59%. Proximate results ranges from (3.18± 3.13%) moisture, (1.87 ± 1.81%) ash, (3.79 ± 2.96%) protein, (0.73 ± 0.56%) lipid, (3.10 ± 1.84%) fiber, (96.87 ± 96.81%) dry matter and (92.35 ± 90.90%) nitrogen free element (NFE). Mineral results ranges from 40.88 ± 28.65ppm, 36.56 ± 18.53ppm, 48.72 ± 29.78ppm, 30.4 ± 17.17ppm, 2.339 ± 1.489ppm, 0.27 ± 0.20ppm, 0.20 ± 0.12ppm, 0.087 ± 0.033ppm, 0.66 ± 0.40ppm for Ca, Mg, Na, K, Fe, Mn, Cu, Zn and PO_4 respectively. The presence of these phytochemicals, proximate and mineral elements in the plant could be part of the contributing factors which suggest the use of the plant for various therapeutic applications. This also indicates that *Bryophyllum pinnatum* plant is a good source of human nutrition and should be included as dietary supplement.

Keywords: *Bryophyllum pinnatum*, phytochemicals, proximate, minerals, medicinal plant

Introduction
Plants play important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bio- active substances like antioxidants, hypoglycemic and hypolipidemic factors. Plants have invariably been exemplary source of drugs and a number of currently available drugs happen to be derived directly or indirectly from them. This natural source has received considerable attention for discovery and development of leads as new drug molecules, because of its diversity. Rural people depend on herbal and traditional medicines to cure their diseases as medicinal plants are easily available in their surroundings and have low cost with increased efficacy and reliability [1]. According to World Health Organization (WHO) 80% of the population rely on traditional medicine as a source of primary health care needs [2].

The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed [3]. Modern-day pharmacopoeia however contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated form plants. Involvement in medicinal plants as a re-budding health assistance has been fuelled with the rising charges of prescription drugs in the safeguarding of personalized health and well being and the bio prospecting of new plant derived drugs [4]. Medicinal herbs are a source of chemical compounds such as alkaloids, glycosides, saponin, oleoresins, sesqueterpine, lactones and oils [5]. These biologically active ingredients are used for the prophylactic purposes and for the different infectious diseases [6]. Due to the presence of medicative properties, medicinal plants have been used in wide area of the world. Many diseases like malaria, epilepsy, diarrhea, dysentery, fungal and bacterial infections have been treated by folklore medicines [7].

B*ryophyllum pinnatum* belongs to the family Grassulaceae an erect, succulent, perennial shrub that grows about 1.5m height and reproduced from seeds and also vegetatively from leaf bubbils [8]. It is an introduced ornamental plant that is now growing as weed around plantation crops [9]. Bryophyllum pinnatum is commonly known as air plant, never die, miracle leaf, love

plant. It is used in folk medicine in tropical Africa, tropical America, India, China, and Australia. It is well known for its wound healing and haemostatic properties. Traditionally, it is used for medicinal purpose for treatment of various ailments viz. anthelmintic, immunosuppressive, hepatoprotective, anti-nociceptive, anti-inflammatory, anti-diabetic, nephroprotective, antioxidant, antimicrobial, analgesic, anticonvulsant, neuropharmacological and antipyretic activities [10]. In South Eastern Nigeria, this herb is used to facilitate the dropping of the placenta of a newly born baby [9]. The plant leaf is mildly exposed to heat and the juice extracted and applied to the baby's placenta on daily basis. The crushed leaves as well as the extracted juice are mixed with palm oil and rubbed on abscesses [8]. It is usually applied externally.

The aim of this study is to determine the phytochemical, proximate and mineral compositions of *Bryophllum pinnatum* medicinal plant.

Materials and Methods

Collection of Plant material

Bryophyllum pinnatum plant was collected from vegetative garden in Yenagoa, Bayelsa State. The plant parts were sundried, pulverized and stored in an airtight container for laboratory analysis.

Qualitative Phytochemical Screening

Phytochemical screening of the extracts was carried out by a procedure that was based on those earlier reports by [11-12, 7].

Test for saponins

To 0.5g of extract, 5ml of distilled water was added in a test tube and the solution shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for terpenoids

0.5g of the extract was dissolved in 1ml of chloroform and 1ml acetic anhydride added, followed by the addition of 2ml of concentrated H_2SO_4. Terpenoids was indicated by formation of reddish violet colour.

Test for tannins

About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration.

Test for cardiac glycosides (keller-killiani test)

To 0.5g of extract dissolved in 5ml water was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for flavonoids

Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for alkaloids

Extract was dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Quantitative Phytochemical Analysis

Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, Phenols, Saponin, Flavonoids and Terpenoids

Total Tannins Content Determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

Total Phenol Content Determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

Total Alkaloid Content Determination

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total Flavonoid Content Determination

The total flavonoids content of samples was determined by following the Aluminum chloride method. Plant concentrate was mixed with distilled H_2O and $NaNO_2$ solution. After 6 min, $AlCl_3$ solution was added and enabled to stand for 6 min, NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and enabled to stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of extracts.

Total Saponin Content Determination

Test extract were dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

Total Terpenoid Content Determination
The extract (1g) was marcarated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

Methods for Proximate Analysis
The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were determined in powdered *Bryophyllum pinnatum* and *Vernonia amygdalina* plants using the standard methods of the Association of Official Analytical Chemists [13] while Dry Matter and Nitrogen Free Element contents was calculated based on the net difference between the other nutrients and the total percentage composition.

Estimation of ash
About 2g of the sample was weighed and taken in a vitreosil basin. The basin was heated in a low flame at the beginning till no fumes were given off by the charred mass. It was broken by a glass rod carefully and burnt in a muffle furnace at 550- 600°C for 4-5 hrs. The muffle was allowed to cool to 150°C. The basin was then cooled in a desiccator and the ash content was then weighed. The total ash was calculated as follows:
% of total ash = weight of the ash ×100 / weight of the sample

Estimation of moisture content
Fresh sample materials were taken in a flat bottom dish and kept overnight in a hot air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of crude protein (Micro-Kjeldahl Method)
Digestion: About 2gm of sample was taken in a Kjeldahl flask, 10gms of sodium sulphate and 0.5 gm of copper sulphate was added and mixed well. A few glass beads were added into the flask to prevent spurting while heating. Then 25 ml of concentrated H_2SO_4 was added and then heated for 15-20 mins in inclined position. The solution was boiled until a greenish colour was obtained. It was allowed to cool.

Distillation
About 100 ml of distilled water was added to the Kjeldahl flask, shaken properly and transferred it 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 was 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 and added 1 drop of phenolphthalene and 10-15 ml 40% NaOH. The distillation was carried out atleast for 5-10 mins until ammonia was free from aliquot. Titration: The distillation product was then titrated against N/10 H_2SO_4

Calculation is done as follows:

$$\% \text{ of Nitrogen} = \frac{\text{ml of N/10 } H_2SO_4 \text{ used up} \times 250 \times 0.0014 \times 100}{\text{Volume of aliquot} \times \text{gm of the substance taken}}$$

% of crude protein=% Nitrogen ×6.25

Estimation of crude Lipid (Ether extract)
Five gm of dry sample was weighed on a piece of glazed paper and transferred into an extraction thimble. The thimble was introduced into soxhlet extractor over a pad of cotton wool, so that top of the thimble is well above the top of the siphon. A clean dry flask was taken, weighed and was fitted with the extractor. Ether was poured along the side of the extractor until it begins to siphon off. Then another half-a siphonful of ether was added. The equipment thus assembled with the flask was placed on a water bath at 60-80°C and the extractor was connected with the condenser. Cool water circulation was started in the condenser and allowed the extraction for 8 hr. Then the thimble with the material was removed from the extractor. The apparatus was assembled again and heated on a water bath to recover all the ether from the receiver flask. The receiver flask was disconnected and dried it in a hot air oven at 100°C for 1 hr, cooled and weighed.

$$\% \text{ of Ether extract} = \frac{(\text{Wt. of oil flask with ether extract} - \text{Wt. of the oil flask}) \times 100}{\text{gm of the substance taken}}$$

Determination of crude fibre
About 2 gm of moisture and fat free sample was weighed and transferred to the spout less one litre beaker. Thereafter, 200 ml 1.25% H_2SO_4 was added. The beaker was placed on hot plate and allowed to reflux for 30 mins, timed from onset of boiling. The content was shaken after every 5 min. The beaker was removed from the hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 mins. Again filtered and the residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110 °C and weighed. The residue was ignited in muffle furnace at 550-600 °C for 2-3 hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

$$\% \text{ of Crude Fiber} = \frac{(\text{Wt of the crucible with dry residue} - \text{Wt of crucible with ash}) \times 100}{\text{gm of the substance taken}}$$

Procedure for Mineral analysis
Estimation of Fe, Zn, Mg, Mn, Na, K and Cu
For this study, 0.5 gm of powdered dried sample was taken in a crucible and converted to ash in the muffle furnace at 580°C for 3 hrs. After cooling in a desiccators 10 ml of concentrated Nitric acid, 4 ml of Perchloric acid and 1ml of Sulphuric acid was added and digestion at high temperature was carried out until the content became clear, then the tube was cooled and the solution was transferred quantitatively to 50 ml volumetric flask and the final volume was adjusted to 50 ml by adding distilled water. The solution was used for determination of Fe, Zn, Mg, Mn, Na, K and Cu through the atomic absorption spectrometry (AA203D). Calcium and Phosphorous estimation were done as per method described by Oyodele [14].

Results
Qualitative and Quantitative Phytochemical Analysis of Never Die plant
Qualitative phytochemical compositions of never die plant (Leaves, Stems and Roots) shows the presence of Alkaloid, Tannin, Saponin, Flavonoid, Terpenoid, Glycoside and

Phenols but flavonoids were absent on the roots as shown in table 1. While quantitative phytochemical composition of never die plant (Leaves, Stems and Roots) was highest in Alkaloids, Tannins and Phenols but lowest in flavonoids as shown in Fig. 1

Table 1: Qualitative Phytochemical Composition of Never Die Plant

Plant Samples	Phytochemical Properties						
	Alkaloid	Tannin	Saponin	Flavonoid	Terpenoid	Glycoside	Phenols
Leaves	+++	++	+	++	++	+++	++
Stems	++	+	++	+	++	+	+
Roots	+	+	+	-	++	++	++

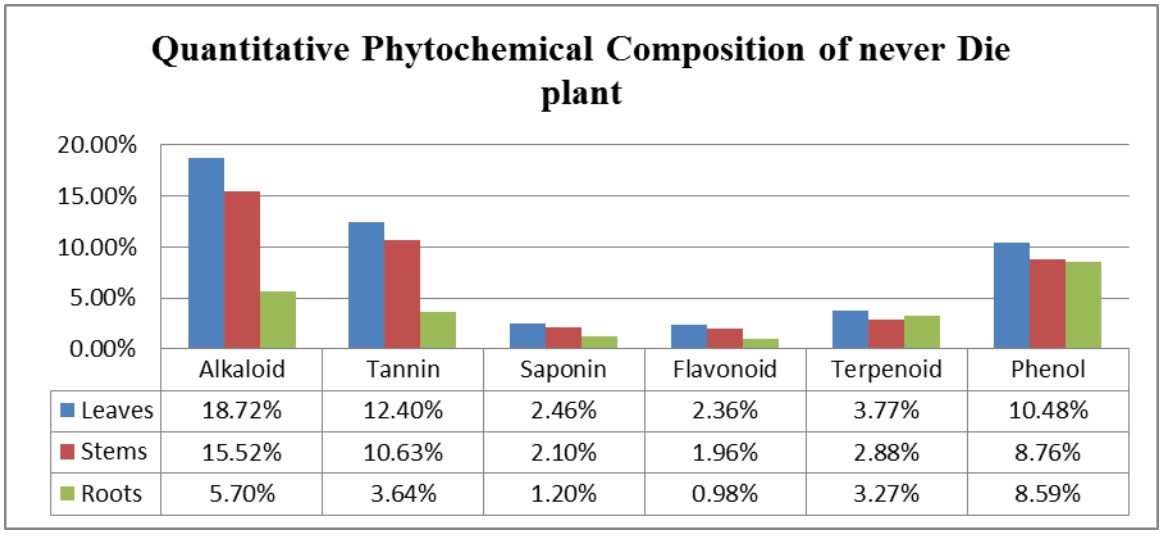

Fig 1: Quantitative Phytochemical Composition of Never Die plant

Proximate analysis of Never Die plant

Proximate compositions of the leaves, stem and roots of Never Die plant was highest in protein and Moisture contents and lowest in Lipid and ash contents as shown in Fig. 2.

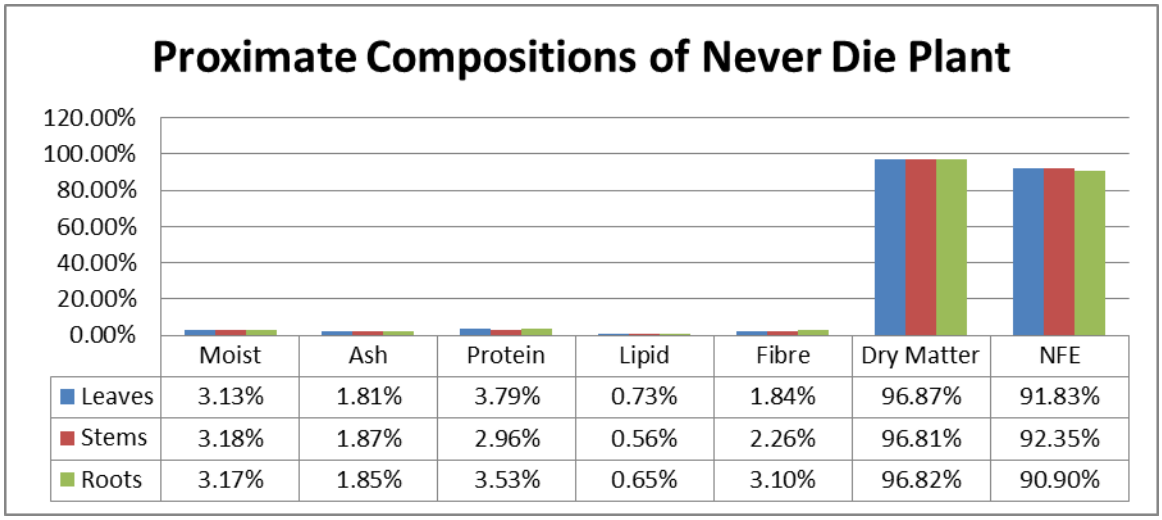

Fig 2: Proximate Compositions of Never Die Plant

Mineral contents of Never Die Plant

Mineral contents of Leaves, stems and roots of never die plants shows highest in calcium, sodium and magnesium and Lowest in zinc, copper and manganese as shown in table 2.

Table 2: Mineral Content of Never Die Plant

Plant Samples	Minerals (ppm)								
	Ca	Mg	Na	K	Fe	Mn	Cu	Zn	PO_4
Leaves	38.47	18.53	29.78	17.17	2.212	0.20	0.12	0.074	0.40
Stems	28.65	21.46	30.98	18.49	1.489	0.27	0.18	0.055	0.53
Roots	40.88	36.56	48.72	30.4	2.339	0.24	0.20	0.087	0.66

Discussion

Phytochemical analysis is very useful in the evaluation of some active biological compound of some medicinal plants. The qualitative phytochemical analysis of the roots, leaves and stems of *Bryophyllum pinnatum* were carried out and alkaloid, glycoside, tannin, phenols, saponin, terpenoid and flavonoids was present on the roots, stems and leaves of the plant samples but flavonoid was absent on the roots. Quantitatively, alkaloids were present in appreciable amount (18.72 ± 5.70%). Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants. Pure

isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties [15]. Alkaloids tend to be organic and natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. They are found in reducing stress and depression symptoms. Alkaloids tend to be poisonous when taken in bulk amount due to their stimulatory effects, producing excitation associated with cell and nerve disorders [16-17].

The tannin content (12.40 ± 3.64%) in the plant samples implies that the leaves, roots and stems of *Bryophyllum pinnatum* have high astringent properties. Tannins quicken the healing of wounds and inflamed mucous membranes [18]. Tannins are water soluble phenolic compounds which precipitate proteins from aqueous solution. They occur in all vascular plants. Tannins bind to proteins making them bio-unavailable [19-21]. The value in this study is in agreement with the studies associated with other researchers in the same field [22-26]. Saponin content (2.46 ± 1.20%) suggests the usefulness of the plant as a potential fertility agent. The saponin level is however low, when compared with the results from other works [26-27, 23]. Saponins at low levels < 10% are said to be safe and non-toxic. Saponins are glycosides containing polycyclic aglycone moiety of either C_{27} steroid or C_{30} triterpenoids attached to a carbohydrate sugar. High Saponin levels have been associated with gastroenteritsis, manifested by diarrhea and dysentery [28].

The presence of flavonoids in appreciable amount (2.36 ± 0.98%), inferred that the plant samples has the biological functions such as anti-oxidation, and protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumour [29, 18]. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, and have strong anticancer and anti-ulcer activity and protection against the different levels of carcinogenesis [29]. Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure [30].

The moisture content was (3.18 ± 3.13%) indicating that the plant is susceptible to spoilage. The ash content (1.87 ± 1.81%) is an indication of the level of inorganic elements such as calcium, zinc, magnesium, copper, and potassium in the plant samples. The protein content was (3.79 ± 2.96%) and readily available as a macronutrient. Protein is an essential component of human diet needed for the replacement of tissues and for the supply of energy and adequate amount of required amino acids. Protein deficiency causes growth retardation, muscle wasting, oedema, abnormal swelling of the belly and collection of fluids in the body of children [31]. High contents of protein in plants are for building and repairing of body tissues, regulation of body processes and formation of enzymes, hormones and antibodies that enable the body to fight infection [32]. The crude fibre content (3.10 ± 1.84%) which aid digestion, absorption of water from the body and bulk stool. Fibre softens stool and therefore, prevents constipation [33]. The plant may therefore be useful in the control of body weight, blood cholesterol and protection against colon cancer. The lipid content (0.73 ± 0.56%) of the plant samples was low, and it can therefore be recommended as part of weight reducing diets. Low lipid foods are said to reduce the level of cholesterol and obesity [34].

The calcium content (40.88 ± 38.47ppm) was high in the plant samples. Calcium helps in the regulation of muscle contraction required by children, infants and foetuses for bones and teeth development [35]. Normal extracellular calcium concentration is necessary for blood coagulation and for the integrity, intracellular cement substance [36]. It also helps in the development of strong bone and teeth. Potassium content (30.4 ± 17.17ppm) was also high in the plant samples, and this is in agreement with many reports that potassium is the most abundant mineral in Nigerian agricultural products [37]. Potassium helps to maintain body weight and regulate water and electrolyte balance in the blood and tissues (National Research Council, NRC), [38]. The concentration of sodium in the sample was (48.72 ± 29.78 ppm), and supports the claim by the natives that the plant is useful in the treatment of heart related diseases. Excess sodium consumption leads to hypertension [38].

Zinc is said to be an essential trace element for protein and nucleic acid synthesis and normal body development [39]. Zinc also stimulates the activity of vitamins, and the formation of red and white blood cells [40]. Zinc plays a role in improving male fertility. The presence of zinc in the plant could mean that the plant can play valuable roles in the management of diabetics which result from insulin malfunction [41-42]. Iron is said to be an important element in the diet of pregnant women, nursing mothers, infants, convalescing patients and the elderly to prevent anaemia and other related diseases [43]. The magnesium content of the plants was found to be 36.56 ± 18.53ppm. Magnesium plays fundamental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acids. It plays a significant role in the intestinal absorption of electrolyte in the body. Its deficiency in man includes severe diarrhoea and persistent migraines [44].

Copper level in the plant samples under study ranged from 0.20 to 0.12ppm. Copper is involved in the formation of red blood cells and synthesis of haemoglobin. It has a role in energy production, wound healing, skin and hair color as well. Copper is also involved in stimulating body defence system. In combination with Zinc, it plays a role in superoxide dismutase activity and the removal of oxygen free radicals [45]. Consumption of manganese-containing foods is believed to support the immune system. Manganese regulates blood sugar levels, the production of energy and cell reproduction. Deficiency in manganese may result in birth defects if an expectant mother does not get enough of this important element [46].

Conclusion

The result of this research work shows that the leaves, roots and stems 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, anti-inflammatory, 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. We believed that the data provided by us will be helpful to explore more medicinal plants. Further

researches are necessary to determine precisely the different constituents' present especially vitamin contents.

References

1. Krishnaraj AV, Rao TV, Sundararaju D. Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay. Int J Appl Sci Eng. 2005; 2:125-34.
2. Mukhopadhyay S. Conservation, protection and biodiversity of medicinal plants. In Prospects of Medicinal Plants, (Eds.) Gautam, P. L. *et al*. Indian Society for Plant Genetic Resources, New Delhi, 1998, 15-28.
3. UNESCO. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO, Paris, France, 1996, 129.
4. Lucy H, Edgar JD. Medicinal Plants: A reemerging Health aid. Electronic. Journal of Biotechnology. 1999; 2(2):1- 15.
5. Singh AP. Promising Phytochemicals from Indian Medicinal Plants. Ethnobotanicals Leaflets. 2005, 1 Article 18.
6. Anpin Raja RD, Jeeva S, Prakash JW, Johnson M, Irudayaraj V. Antibacterial Activity of Selected Ethnomedicinal Plants from South India. Asian Pac. J Trop. Med. 2011; 4:375-378.
7. Sofowora A. Research on Medicinal Plants and Traditional Medicine in Africa. J Altern. Complement. Med. 1996; 2(3):365-372.
8. Agoha RC. Medicinal Plant of Nigeria. Offset Drakkerji. Faculfcitder Wiskunde in Natuurwetenschappen, the Netherlands. Science Bulletin. 1974; 4:33-41.
9. Dalziel JM. The useful plant of West Tropical Africa. Journal Science, 1995; 28:53-415.
10. Muhammad I, Samoylenko V. Antimalarial Quassinoids: Past, Present and Future. Expert Opinion on Drug Discovery. 2007; 2:1065-1084.
11. Harborne JB. Phytochemical methods- A guide to modern techniques of plant analysis. 2nd edition, Chapman and Hall, London, 1984.
12. Trease GE, Evans WC. Pharmacognosy. 11th edition, Brailliar Tiridel Can. Macmillan Publishers, UK, 1989.
13. AOAC, Official methods of analysis, 18th edition, Association of Official Analytical Chemists International, Gathersburg, MD U.S.A, 1990.
14. Ayodele AE. Methods in plant analsysis. Journal of Science. 2001; 10:134-136.
15. Stray F. The Natural Guide to Medicinal Herbs and Plants. London: Tiger Books International, 1998, 12-16.
16. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993; 342:1007-1011.
17. Ezeabara CA, Okeke CU, Aziagba BO. Flavonoid content of Citrus species grown in Awka, Anambra State, Southeastern Nigeria. Int. J Agric. Biosci. 2013; 2:103-107.
18. Farquar JN. Plant Sterols, their biological effects in human. Handbook of Lipids in Nutrition BOCA Rotan HL CRC Press, 1996, 101-105
19. Bagepallis S, Narasinga R, Tatinemi P. Tannin contents of foods commonly consumed in India and Its influence on ionisable iron. J Sci. Food Agric, 1993; 33:89-96.
20. Aleto VA, Adeogun OA. Nutrients and antinutrients components of some tropical leafy vegetables. Food Chem. 1995; 54(4):375-379.
21. Sotelo AE, Contrerar S, Flores S. Nutritional value and content of antinutritional compounds and toxics in ten wild legumes of Yucatan peninsula. Plant Food Hum. Nutri, 1995; 47:115-123.
22. Chinma CE, Igyor MA. Micronutrients and anti-nutritional contents of selected tropical vegetables grown in South East, Nigeria. Nigeria Food Journal, 2007; 25(1):111-116.
23. Ekop AS. Determination of Chemical Composition of *Gnetum africanum* (AFANG) seeds. Pakistan J Nutr. 2007; 6(1):40-43.
24. Abidemi TA, Adebayo OJ, Idowu O, Agbotoba MO. Nutrient content and anti-nutritional factors in shea butter (*Butryospermum parkii*) leaves. Afr. J Biotechnol. 2009; 8(21):5888-5890.
25. Amoo IA, Aasie VN, Kolawole OO. Proximate Composition, Nutritional valuable Minerals, protein. Functional Properties and Anti-Nutrient contents of Mucuna Preta, Mucuna Ghana and Mucuna Veracruz Mottle. Pak. J Nutr. 2009; 8(8):1204-1208.
26. Nkafamiya II, Modibbo UU, Mariji AJ, Haggai D. Nutrient content of seeds of some wild plants. Afr. J Biotechnol. 2007; 6(14):1665-1669.
27. Umaru HA, Adamu R, Dahiru D, Nadro MS. Levels of antinutritional factors in some wild edible fruits of Northern. Nigeria. Afr. J Biotechnol. 2007; 6(16):1935-1938.
28. Awe IS, Sodipo OA. Purification of saponins of root of Blighia Sapida Niger. J Biochem. Mol. Biol, 2001; 16(3):201-204.
29. Okwu DE. Phytochemicals and vitamin content of indenous species of southeastern Nigeria. Journal of Sustaining Agricultural Environment. 2004; 6:30-37.
30. Radford DJ, Gillies AD, Hinds JA. Naturally occurring cardiac glycoside. Med. J Aust. 1986; 144:540-544.
31. Mounts TL. The Chemistry of Components (2nd ed.). Royal Society of Chemistry, 1972.
32. Helsper J, Hoogendijk M, Van Norel A, Burger-Meyer K. Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannin. J Agric Food Chem. 1993; 41:1058-1061.
33. Ayoola PB, Adeyeye A. Proximate Analysis and Nutrient Evaluation of Some Nigerian Pawpaw Seeds Varieties. Science Focus. 2009; 14(4):554-558.
34. Gordon MN, Kessel M. Perspective in Nutrition (5th ed.). Ohio, New York: McGraw Hill Company. 2002; 257-81.
35. Margaret L, Vickery B. Plant Products of Tropical Africa. Macmillan in College ed. London, 1997.
36. Okaka JC, Okaka ANC. Food composition spoilage and shelf life extension. Ocjare Academic Publisher, Enugu, Nigeria. 2001, 54-56.
37. Afolabi G, Oluwade A, Tunde O. Estimation of Proximate and Mineral Composition of Some Tropical Crops. African Agricultural Journal. 1995; 21:103.
38. NRC. National Research Council Recommended Dietary Allowance, National Academy Press, Washington D. C, 1989.
39. Melaku U, Clive EW, Habtamon F. Content of zinc, Iron, Calcium and Their Absorption Inhibitors in Ethiopia. Journal of Food Composition Analysis. 2005; 18:803-817.

40. Claude B, Paule S. The Manual of Natural Living (1st ed.). Biddles Ltd., Guildford, Surrey, 1979, 98-101.
41. Pal S, Nag Khaudhari AK. Preliminary studies on the anti-inflammatory and analgesic activities of *Bryophyllum pinnatum*. Med. Sci. res. 1989; 17(17):561-562.
42. Okwu DE. Evaluation of the chemical composition of indigenous species of south Eastern Nigeria. Global J. Pure Appt. Sci. 2001; 7:455-459.
43. Oluyemi EA, Akilua AA, Adenuya AA, Adebayo MB. Mineral Contents of Some Commonly Consumed Nigerian Foods. Science Focus. 2006; 11(1):153-157.
44. Appel LJ. Nonpharmacologic Therapies that Reduce Blood Pressure. A fresh Perspective. Clin. Cardiol, 1999; 1111-1115.
45. Guo JY, Han CC, Liu YM. A contemporary treatment approach to both diabetes and depression by *Cordyceps sinensus*, rich in vanadium. Evident Based Complementary Alternative Medicine. 2010; 7:387-389.
46. Anhwange BA, Ajibola VO, Oniye SJ. Chemical Studies of the Seeds of *Moringa oleifera* and *Deuterium microcarpum*. Journal of Biological Sciences, 2004; 4(6):711-715.