PROXIMATE ANALYSIS, MINERAL COMPOSITIONS AND IN-VITRO INVESTIGATION OF ANTIDIABETIC POTENTIAL OF METHANOLIC LEAF EXTRACT OF BRYOPHYLLUM PINNATUM

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ABSTRACT

Bryophyllum pinnatum has generated a great deal of interest in the scientific world due to its numerous benefits. The proximate content, mineral composition, and antidiabetic potential of the methanolic leaf extraction were evaluated. The proximate content was evaluated in a fresh sample to determine the ash, carbohydrate, fat, fiber, protein, and moisture using the method of the Association of Analytical Chemists (AOAC), and mineral composition was done using an atomic absorption spectrophotometer (AAS). The in vitro method was used to determine the antidiabetic potential of the leaf by evaluating the inhibitory activity of the leaf extract on alpha-amylase and alpha-glucosidase, respectively. The result shows that Bryophyllum pinnatum contains (0.63 ± 0.05%) ash, (3.72 ± 1.37%) carbohydrate, (1.13 ± 0.32%) lipid, (3.90 ± 0.26%) fiber, (2.33 ± 0.472%) protein, and (88.08± 1.34%) moisture in fresh samples. The result of the mineral content revealed that Bryophyllum pinnatum is a good source of minerals such as ‘Fe, Zn, Cu, Mg, Na, K, Ca, Mn, Se, and Co. Furthermore, the antidiabetic potential of the leaf showed that at various concentrations of the leaf extract, there was an appreciably level of inhibition on both alpha-amylase and alpha-glucosidase, respectively. This research shows that Bryophyllum pinnatum leaf is a good source of human nutrition and should be included as a dietary supplement. The leaf can also be used in the treatment or management of diabetic-related issues. *[African Journal of Chemical Education—AJCE 14(3), July 2024]*

INTRODUCTION

The use of traditional medicines and medicinal plants has been broadly used in many developing countries as remedial agents for the maintenance of health and other benefits.[31] Medicinal herbs are a source of chemical compounds such as alkaloids, glycosides, saponins, oleoresins, sesqueterpine, lactones, and oils.[27] These biologically active ingredients are used for prophylactic purposes and for different infectious diseases.[20] Many diseases, like malaria, epilepsy, diarrhea, dysentery, and fungal and bacterial infections, have been treated by folklore medicines. [28] Studying the biological and pharmacological properties of medicinal plant extracts is a rational approach in our quest for new drugs.[32] The use of medicinal plants is more common among people who have little or no access to modern medical assistance. Hence, a number of studies have been published with the aim of evaluating the anti-inflammatory activity of the organic or aqueous extracts of these plants, either on human monocytes in vitro or on rodents in vivo.[17].

Bryophyllum pinnatum belongs to the family Crassulaceae and is commonly known as the Canterbury Bells, love plant, miracle leaf, and life plant.[2] It is a succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and vegetatively from leaf bulbils. It has a tall, hollow stem and freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers. This plant can easily be propagated through stems or leaf cutting.

It is an introduced ornamental plant that is now growing as a weed around plantations. B. pinnatum is used in ethnomedicine for the treatment of earaches, burns, abscesses, ulcers, insect bites, whitlows, diarrhea, and cithiasis.[24] B. Pinnatum is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides, and lipids.[15].

In south-eastern Nigeria, Bryophyllum pinnatum is used to facilitate the dropping of the placenta of newborn babies.[7] The lightly roasted leaves are used externally for skin fungus. The leaf infusions are an internal remedy for fever. B. pinnatum is also used to expel worms, cure acute and chronic bronchitis, pneumonia, and other forms of respiratory tract infections such as asthma. The plant is considered a sedative, wound-healer, diuretic, and cough suppressant. The plant is also employed for the treatment of kidney stones, gastric ulcers, and edema of the legs [24] and is also widely used in the Ayurvedic system of medicine as an astringent, analgesic, carminative, and useful in nausea and vomiting.[15].

The aim of this research was to determine the anti-diabetic properties, some mineral compositions, and proximate constituents of Brayophyllum pinnatum leaf.

MATERIALS AND METHODS

Fresh leaves of *Bryophyllum pinnatum* were collected from a vegetation garden in Bonsaac, Lat 6.1829°N, Asaba, Delta State, Nigeria. The plant was identified and authenticated by Dr. C. J. Ukpaka, a botanist in the biological department at Chukwuemeka Odumegwu Ojukwu University in Anambra State.

Sample Digestion for Mineral Analysis

The plant samples were dried and burned to ash using a muffle furnace at 700 °C. 1 gram of the ash was weighed into a 250 ml beaker, and 10 ml of 1:1 HNO_3 was added, covered with a watch glass, and heated in a water bath for 1 hour. 2 ml of distilled water and 3 ml of hydrogen peroxide were added, and heating continued in the water bath at 99.9 degrees. After the peroxide effect stopped, 1 ml of peroxide was added at a time until a total of 8 ml was added. The beaker was brought out, and 10 ml of distilled water and 5 ml of concentrated HCl were added. The mixture was put back on the water bath and heated for 15 minutes. After 15 minutes, it was filtered into a 100 ml volumetric flask and made up with deionized distilled water.

Sample Extraction

Fresh *B. pinnatum* leaves were dried in an electrothermal hot air oven (Eppendorf, Germany) at 35 ^{o}C. Then, the dried sample was pulverized. 230 g of the pulverized sample was placed in an airtight container. 2.0 L of methanol and H_2O in the ratio of 1:1 v/v was added and stirred occasionally for 2 hours. The mixture was allowed to stand for 48 hours. Thereafter, the mixture was sieved using a mucilin cloth and filtered with Whatman filter paper size No. 1. The filtrate was placed in a hot water bath (Stericox, India) at a temperature of 40 ^{o}C to remove the extracting solvent and obtain a crude concentrate of the extract.

Proximate Analysis: Proximate analysis (moisture, ash, protein, carbohydrate, and lipid content) was determined using the standard method.[4]

Mineral Content Analysis:

Analysis of the digested sample was carried out using an atomic absorption spectrophotometer and a flame photometer. The working standard solution of concentrations (0, 1, 5, 10, 15, 20, 25, and 100 ppm) was prepared from a standard stock solution of 1000 ppm. The tests were performed in triplets, and the concentrations of minerals in the sample were determined by the standard plot.[1]

Estimation of α-Glucosidase Inhibitory Activity

Glycosidase inhibition was assayed as described by [9] with a slight modification. The enzyme was derived from the brushboard of the rat small intestine. The rat was decapitated, and the intestines were removed. The entire intestine was homogenized with four parts of cold distilled water. The homogenate was centrifuged at 4000 rpm for 10 minutes, and the supernatant was used to determine the α-glucosidase activity. A known volume (10, 20, 30, and 40 ml) of the extract solution (0.1%) was added to 50 ml of diluted supernatant of small intestinal enzymes (dilution factor = d). The solution was mixed well and incubated at 37 ^{o}C for 30 minutes. 100 ml of substrate-buffer solution (0.056 M maltose in 0.1M sodium maleate buffer, pH 6.0) was added and mixed well. After exactly 60 minutes from the first incubation, the reaction was interrupted by incubating at 25 ^{o}C for 5 minutes, and then 20 ml of distilled water was added and stirred. At the end of the reaction, the absorbance of the mixture was measured at 405 nm using a spectrophotometer. A reagent blank was prepared in the same manner without the leaf extract and incubated at 37 ^{o}C.

Estimation of α-Amylase Inhibitory Activity

Amylase inhibition was assayed as described by [19] with a slight modification. In the preparation of the blank, 2.5 ml of buffered substrate and 0.1 ml of intestinal homogenate were added in a clean test and incubated at 37 ^{o}C for 30 minutes. After the incubation, 2.5 ml of iodine solution

was added to the mixture, and the mixture was allowed to react completely. At the end of the reaction, the absorbance of the mixture was measured at 540 nm using a spectrophotometer.

For the preparation of the test sample, 2.5 ml of buffered substrate, 0.1 ml of intestinal homogenate, and 2 ml of 31.25 µg/ml of the leaf extract were added to a clean test tube and incubated at 37 °C for 30 minutes. After 2.5 ml of iodine solution was added to the mixture, the mixture was allowed to react completely. At the end of the reaction, the absorbance of the mixture was measured using a spectrophotometer. The above procedure was repeated with different concentrations of the leaf extracts (60.50, 125, 250, and 500 µg/ml), respectively, and their corresponding absorbance were measured accordingly.

RESULTS

Table 4.1 Proximate analysis *Bryophyllum pinnatum* fresh leaves

S/N	Parameter	% Content
1	Ash	0.63±0.05
2	Moisture	88.08±1.34
3	Crude fibre	3.9±0.26
4	Crude protein	2.33±0.47
5	Crude lipid	1.33±0.32
6	Carbohydrate	3.72±1.37

In the proximate analysis, the fresh leaves possess appreciable levels of carbohydrates, protein, fat crude, fiber, ash content, and very high moisture content. The findings of the proximate chemical composition are summarized in Table 4.1.

Table 4.2: Mineral compositions of *Bryophyllum pinnatum* fresh leaves

S/N	Element	Concentration in ppm
1	Magnesium (Mg)	8.41 ± 0.36
2	Manganese (Mn)	7.74 ± 0.35
3	Sodium (Na)	6.24 ± 0.69
4	Calcium (Ca)	7.81 ± 0.98
5	Potassium (K)	11.74 ± 0.12
6	Iron (Fe)	6.53 ± 0.16
7	Zinc (Zn)	14.30 ± 0.06
8	Copper (Cu)	4.67 ± 0.35
9	Selenium (Se)	2.83 ± 1.0
10	Cobalt (Co)	0.43 ± 0.09
11	Cadium (Cd)	0.23 ± 0.08

The mineral contents of *Bryophyllum pinnatum* leaves were investigated in a dry sample. The leaf samples displayed significant values for all the macro- and micro elements analyzed in this study. The results are presented in Table 4.2.

Table 4.3: Estimated α-amylase % inhibition activity of *Bryophyllum pinnatum* leaf

S/N	**Concentration (µg/ml)**	**% Inhibition**
1	31.25	28.94 ± 4.10
2	60.50	26.36 ± 3.43
3	125	25.91 ± 1.20
4	250	24.24 ± 2.05
5	500	22.12 ± 2.58

α-Amylase inhibition activity of the *Bryophyllum pinnatum* leaf extract was monitored using starch as the substrate. The inhibitions were determined at different concentrations, and the results of inhibition are shown in Table 4.3.

Table 4.4: Estimated α-glucosidase % inhibition activity of *Bryophyllum pinnatum* leaves

S/N	**Concentration (µg/ml)**	**% Inhibition**
1	10	44.97 ± 2.07
2	20	31.57 ± 5.03
3	30	57.40 ± 2.90
4	40	44.62 ± 2.99
5	50	58.66 ± 7.55

The in *vitro* antidiabetic potential of the leaf extract *Bryophyllum pinnatum* was also determined by the α-glucosidase inhibition method, and the results of the percentage inhibition at different concentrations are displayed in Table 4.4.

Discussion

Proximate composition analysis: The result in Table 4.1 depicts the percentage compositions of both primary metabolites and physicochemical properties of Bryophyllum pinnatum, which were carried out using a fresh leaf sample of the plant. The primary metabolites include protein, carbohydrates, and lipids. The percentage of carbohydrates is 3.72 ± 1.37 %; protein has its own percentage of 2.33 ± 0.47 %, with an appreciable percentage of lipid, which is 1.33 ± 0.32 %. Assessment of the primary metabolites revealed that the plant leaves contain varied quantities of carbohydrates, proteins, and lipids. Recent findings, which involve, [18] suggested that carbohydrates > protein > lipids in the leaf extract of Bryophyllum pinnatum. The determination of the primary metabolites has shown substantial nutritional value for the plant.[14]

The crude fiber contains 3.90 ± 0.26 %, the ash content is 0.63 ± 0.05 %, and the very high moisture content is 88.08 ± 1.34 %, respectively. They are part of the physicochemical properties of the proximate determination of the plant. The test for ash value is of great importance since it identifies the presence of allied matters such as silica, oxalate, etc. The ash content also indicates

that the plant could act as a preservative, a digestive aid, and a source of inorganic minerals.[3] The composition of ash obtained in Table 4.1 is within the recommended range (less than 20%).[13] The high water percentage value of the fresh leaves suggested the presence of polar substances like phenols, tannins, glycosides, e.t.c.[6] This confirms Bryophyllum pinnatum as a good source of these nutrients and a possible dietary value

Mineral composition analysis: Table 4.2 shows the mineral contents of Bryophyllum pinnatum leaf. The results show an appreciable amount of these minerals (calcium, potassium, magnesium, sodium, zinc, manganese, sodium, copper, and iron). Potassium was the most abundant macroelement in the B. pinnatum leaf sample, with a concentration of 11.74 ± 0.12 ppm. Potassium is an essential nutrient that occurs naturally in many foods and is available as a dietary supplement. Potassium is present in all body tissues and is required for normal cell function because of its role in maintaining intracellular fluid volume and trans-membrane electrochemical gradients [29]. Also, the sample contained an appreciable amount of calcium (7.81 ± 0.98). Normal extracellular calcium concentration is necessary for blood coagulation and for the integrity of the intercellular cement substance.[23] It also helps in the development of strong bones and teeth. Zinc was found to be 14.30 ± 0.06 ppm; the presence of zinc in the leaf sample could mean that the leaf can play a vital role in the management of diabetics, which result from insulin malfunction.[24] Other minerals such as Mg,

Mn, Na, Fe, Cu, Se, Co, and Cd were found to be 8.41 ± 0.36, 7.74 ± 0.35, 6.24 ± 0.69, 6.53 ± 0.16, 4.67 ± 0.35, 2.83 ± 1.0, 0.43 ± 0.09, and 0.23 ± 0.08 ppm, respectively. Magnesium plays a crucial role in various physiological processes, including nerve function, muscle contraction, and maintaining a healthy heartbeat. Magnesium, as a trace element, is required for several enzymatic reactions in the body, and it possesses antioxidant properties.[26] Copper was observed to be present in the leaf at a concentration of 4.67 ppm. It is involved in the iron metabolism and collagen synthesis and supports the formation of connective tissues as well as maintaining healthy skin and hair.[30] Iron is a key component of hemoglobin, and it is reasonably present in the leaf, as shown in Table 4.2. Insufficient amounts of iron in the body result in a condition called iron-deficiency anemia, with symptoms of fatigue, weakness, and a reduction in physical and cognitive performance, respectively. Consequently, a lack of minerals in the body system amounts to different pathological conditions.[12]

Table 4.3 shows the results of the anti-inhibition of alpha-amylase. The results obtained showed relatively moderate inhibition activity on the substrate. The extract was able to inhibit α-amylase, which is the enzyme that converts starch to maltose. At various concentrations of the extract, the percentage inhibitions recorded are 28.94, 26.26, 25.91, 24.24, and 22.12%, respectively. These levels of inhibition show that the leaf extract could be used to control diabetic activity since

it was able to inhibit the enzymes to a semi-moderate level, thereby reducing the level of maltose, which would be subsequently converted to glucose that ought to have diffused into the blood stream. Hence, this inhibition has indirectly reduced the glycemic load, which refers to the amount of carbohydrate consumed multiplied by the rate at which the carbohydrate is metabolized and enters the bloodstream. Bryophyllum pinnatum leaves have been widely reported locally for their anti-diabetic activity, and studies have validated this claim in both in vitro and in vivo animal models.[5] [8] [11] [21] [22]

Table 4.4 shows the result of the inhibition of alpha-glucosidase. The results obtained revealed appreciable inhibition activity on the substrate. The extract was able to inhibit α-glucosidase, which is the enzyme that acts on the α-1, 4-glycosidic bond, thereby converting maltose to glucose. At various concentrations of the extract, the percentage inhibitions recorded are 44.97, 31.57, 57.40, 44.62, and 58.66%, respectively. These levels of inhibition show that the leaf extracts could be used to manage diabetic activity since they were able to inhibit the enzymes to a moderate extent, thereby reducing the level of conversion of maltose to glucose. Consequently, this inhibition has indirectly reduced the available blood glucose. According to [10], the hyperglycaemic effect of Bryophyllum pinnatum extract showed a significant ($p < 0.05$) reduction in the glucose level of

alloxan-induced diabetic rats. The result above agrees with the one obtained by [5] which in their study revealed a reduction in blood glucose by the leaf extract.

CONCLUSION

The proximate composition of the methanolic extract of Bryophyllum pinnatun leaf offers valuable insights into its nutritional potential. The results reveal a diverse array of bioactive constituents, including carbohydrates, proteins, fats, and fibers, which collectively contribute to its nutritional value. Also, the results of mineral compositions show that the extracts contain essential elements for human health, such as calcium, potassium, magnesium, iron, zinc, copper, etc. The abundance of minerals in the extract suggests that the plant could be considered a supplementary source of essential nutrients, especially in regions where micronutrient deficiencies are prevalent. The anti-diabetic activity of the methanolic extract of Bryophyllum pinnatum leaves holds promise as a potential natural remedy for managing diabetes. The result from this study suggests that the bioactive compounds present in the extract may contribute to its ability to inhibit both alpha-amylase and alpha-glucosidase, thereby lowering blood glucose levels and improving insulin sensitivity.

Competing interest

The authors declared that they do not have competing interest

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