PROXIMATE ANALYSIS, PHYTOCHEMICAL PROPERTIES AND ANTIFUNGAL ACTIVITY OF 'MIRACLE PLANT', *BRYOPHYLLUM PINNATUM* (LAM.) OKEN

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ABSTRACT

The leaf and root of *Bryophyllum pinnatum* (Lam.) Oken were evaluated for proximate composition, vitamins, amino acids, phytochemicals, mineral elements, micro-morphology and its antifungal activities on some selected fungal species obtained from diseased *Arachis hypogaea* seeds. These were assessed following standard procedures. The study showed that the protein, moisture, ash, crude fibre and crude fat contents were higher in the root of *B. pinnatum* than in the leaves except for carbohydrate. *B. pinnatum* leaves and roots had water-soluble vitamins (19.45%; 48.22%), fat-soluble vitamins (11.75%; 29.05), essential amino acids (5.324%, 8.318%) and non-essential amino acids (1.189%, 2.355%), respectively. Phytochemicals and mineral concentrations were higher in the roots of *B. pinnatum* than in the leaves. The micromorphology revealed aniocystic stomatal type, the presence of tannins in the petiole and stem as seen in the vascular bundle and secretory cells while starch grains were seen in the root. *Bryophyllum pinnatum* leaf extract inhibited *Rhizopus stolonifer* mycelia growth followed by *Aspergillus flavus* and *Aspergillus niger*. The mycelial growth of these fungi ranged from 2.57 – 3.33 cm compared with the control treatment (8.7 cm). These inherent potentials in *B. pinnatum* could be the reason the plant is recommended for the treatment of various ailments.

Keywords: Antifungal properties, anti-nutrient, *B. pinnatum*, micro-morphology, nutrient

INTRODUCTION

Plants have served several purposes to mankind such as food and medicine for treatment of diseases. According to Sofowara (1993), the active ingredient(s) of most of the commonly used conventional pharmaceutically mass-produced drugs are originally derived from plants. Okwu and Josiah (2006) reported that many native herbal plants have been used to cure diseases and heal injuries. Also, Amor *et al.* (2009) noted that medicinal plants have received substantial recognition because their phytochemicals may lead to new drug discoveries. Falodun and Imieje (2013) noted that herbs are gaining prominence over the world. Few plants or their phytochemical constituents have proven to have medicinal effects by rigorous science (Aminu and Zainab, 2017). Many of these phytochemicals had beneficial long-term

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health effects on humans when the plants were consumed (Aminu and Zainab, 2017). Therefore, medicinal plants have always had great significance in culture, medicine and nutrition of societies in the world, especially in Nigeria as a nation (Okwu and Ekeke, 2003).

Bryophyllum pinnatum Lam. belongs to the family Crassulaceae and is commonly known as Canterbury-bells, love plant, miracle leaf and life plant (Afzal *et al.,* 2013). It is found in the tropical Africa, India, China, America and Australia (Gill, 1992; Devbhuti *et al.,* 2012). It is a succulent perennial plant that grows 1-1.5 m in height and the stem is hollow, four-angled and usually branched. Leaves are opposite, decussate, succulent, 10-20 cm long. The lower leaves are simple, whereas the upper ones have 3-7 foliate and have long petioles. They are fleshy dark-green and are distinctively scalloped and trimmed in red. Leaf blade pinnately compound with 3-5 leaflets, 10-30 cm; petioles 2-4 cm; leaflet blades oblong to elliptic, 6-8 by 3-5 cm, margin crenate with each notch bearing a dormant bud competent to develop into a healthy plantlet (Jaiswal and Sawhney, 2006), apex obtuse. The leaves and leaf juice have been used traditionally as anti-inflammatory, antipyretic, antimicrobial, anti-oxidant, antitumour, antidiabetic, anti-ulcer, antiseptic, antihypertensive, hypocholosterolemic and cough suppressant (Akinpelu, 2008; Ojewole, 2002; Ali *et al.,* 2013). The leaves and bark are not sweet, astringent to the bowels, analgesic, and useful in diarrhoea and vomiting (Quazi *et al.,* 2011). Studies have shown the role of these phytochemicals in some medicinal plants on the central nervous system activities (Dorr *et al*, 1971; Fujimori, 1995; Wakeel, 2004). The leaf extract of *B. pinnatum* showed some significant effects, which indicate substantial antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at different levels. They showed that the leaves of *B. pinnatum* possess antimicrobial properties which can be harnessed for the production of antimicrobial drugs (Obioma *et al.*, 2017). The leaves of *B. pinnatum* contain mineral elements such as Ca, K, Mg,Na, Zn, Cd, Ni, Pb and Fe (Nwali *et al.*, 2014). The potential of *B. pinnatum* to stop bleeding and its use in treating wounds could be as a result of its high calcium content (Okwu and Josiah, 2006).

The aim of this work was to study the antifungal effect, microscopic, phytochemical and mineral content of *Bryophyllum pinnatum.*

MATERIALS AND METHODS

Collection of plant samples: The fresh and matured leaves and roots of *B. pinnatum* (Plate I) were collected between March-April, 2017 from the botanical garden in the main campus of the University of Port Harcourt. The *Bryophyllum* species was authenticated by a taxonomist, Dr. C. Ekeke, in the Herbarium Unit, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and assigned voucher number UPH/V/2671.


Plate 1: *Bryophyllum pinnatum* (Lam.) Oken

Proximate Analysis: Proximate analysis (moisture, ash, protein, carbohydrate and lipid content) was determined using standard method (AOAC, 1990). The crude fibre was determined by subtracting difference from others (100 –moisture + ash + protein + carbohydrate + fat content).

Determination of Vitamins

The extraction and determination of vitamins A, B_2, B_6, B_{12} and E were according to the method described by Okonwu *et al.* (2018a,b) while vitamin C was determined using titrimetric method (Okwu, 2004).

Vitamin A extraction and determination using waters 616/626 HPLC

Plant sample (0.5 g) was weighed into a cornical flask, 20 ml of 0.2 *N* HCl dispensed and allowed to stand for 1.5 hours. The solution was cooled and the pH adjusted to pH 6, using NaOH. Also, 1*N* HCl was added to lower the pH to 4.5. The solution was made up to 50 ml, shaken and centrifuged for 10 minutes at 3000 rpm. The supernatant was separated, 1 ml of acetic acid (CH_3COOH) added and mixed properly. Also, 0.5 ml of 3% H_2O_2 was added and mixed well. Finally, 20 mg of sodium hydrogen sulphate was added and shaken properly. The extract was run on HPLC (Waters 616/626). Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40ºC column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 2, 4, 6, 8 ppm) and determination was carried out at a wavelength of 328 nm.

Vitamin B_2, B_6, B_{12} combined extraction and determination using Waters 616/626 HPLC

Plant sample (2.5 g) was weighed into a set of digestion tubes, and an extraction solution (Ultra-pure water: HCl: 0.1*N* H_2SO_4, in the ratio 5:2:3) dispensed. The tube was warmed at the temperature of 40% for 2 hours, allowed to cool to room temperature and transferred to a set of plastic centrifuged tubes. The

latter was shaken for 10 minutes and centrifuged at 3000 rpm. The supernatant was set in autoanalyser tubes and run on HPLC. Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40^{o}C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6, 0.8 ppm) and determination was carried out at a wavelength range of 240 - 465 nm.

Vitamin E extraction and determination using Waters 616/626 HPLC

Plant sample weighing 0.5 g each was weighed into a set of digestion tubes, 20 ml of diluted hydrochloric acid (HCl) was added and shaken vigorously for 2 hours. The extract was further treated with phosphatase to liberate free vitamin E into the solution. The extract was purified by passing through base exchange silicate alkaline column to remove interfering compound. Thereafter, the extract was stored in a set of vials for analysis using HPLC. Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40^{o}C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6, 0.8 ppm) and determination was carried out at a wavelength of 356 nm.

Determination of Amino Acids using Waters 616/626 (HPLC) Instrument

The sample preparation and determination were carried out in the following four stages according to the procedure described by Okonwu *et al*. (2018a, b).

Phytochemicals

The leaves and roots were rinsed with distilled water to remove dirt and prepared differently to be used for respective analyses: hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid, flavonoids and organic acids. The analyses were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, flavonoid and alkaloid contents of *B. pinnatum* leaves and roots were determined following the method used by Okonwu *et al.* (2017a, b) and Okonwu and Muonekwu (2019) while organic acids were determined according to Okonwu and Akonye (2019) and Okonwu and Muonekwu (2019). Waters 616/626 liquid chromatography was the tool used in determining flavonoids, alkaloids and organic acids content.

Flavonoids (Extraction and Analysis)

Plant samples (1.5 g) each were weighed into a set of extraction tubes and 20 ml of boiled ultra-pure water dispensed into each extraction tube. The set-up was allowed to stand for 1.5 hours and voltexed for 5 minutes. The solution was transferred to a set of centrifuge tubes, shaken for 15 minutes and centrifuged for 5 minutes at 3000 rpm. Thereafter, a set of vials were used to collect the supernatants for determination on water 616/626 HPLC. The conditions for the analysis of flavonoids were as follows: (i) an autosampler (ii) an automated gradient controller (iii) gradient elution HPLC pump (iv) reverse-phase

HPLC column, thermostatically heated in a temperature-controlled room (v) detector by fluorescence (vi) carrier gas: nitrogen gas at flow rate of 60 ml/min (vii) Temperature: Detector- 147ºC; Injector port- 166ºC and Column: 115ºC (viii), Computer facilities for storing data. (ix) Printer for results reporting.

Alkaloids (Extraction and Analysis)

Ten gram (10 g) of plant sample was de-fated, out of which 5 g was weighed into a flask and 100 mL of 12% alcohol added, shaken, filtered and washed with industrial alcohol. The extracted residue was washed into a flask with 50 ml of ammonia water (ultrapure water), heated in boiling water for 20 minutes and allowed to cool. Then, 0.1g of diastase (+ water) was added and maintained at 50-55ºC for 2 hr. It was cooled and made up to 250 mL with ultrapure water, swirled and filtered. The filtrate (200 mL) was mixed with 20 mL hydrochloric acid (sp.g. 1.125) and heated in boiling water for 3 hours. Thereafter, it was allowed to cool, neutralised with sodium hydroxide solution and made up to 250 mL. The sample was shaken, centrifuged and supernatant decanted for determination using water 616/626 HPLC. The conditions of HPLC (Water 616/626) for the analysis of alkaloids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 40 mL/mins (vii) Temperature: Detector- 170ºC; Injector port- 190ºC and Column- 125ºC (viii) Computer facilities for storing data (ix) Printer for results reporting.

Organic acids (extraction and analysis)

Plant sample (5 g) was weighed into 250 ml extraction bottle, 100 ml of ultrapure water was dispensed to cover the sample and placed in a cool environment at 4ºC for 6 days. Then sample materials were squeezed from the extraction bottle through two layers of cheesecloth (or glass wool in a funnel); the liquid was centrifuged at about 2000 rpm for 5 minutes. The sample was placed in corked bottle and stored in a cooled environment. Sample solution (1.5 ml) and 1ml working standard were pipetted into a 10 ml centrifuge tube, mixed and allowed to stand for 30 minutes. Thereafter, the content was centrifuged at 3000 rpm for 10 minutes. The supernatant liquid (1.5 ml) was injected directly on the HPLC column (4% CW-20M 80/120 carbg pack-BDA column) fitted with a flame ionization detector. The conditions for the analysis of organic acids were as follows: (i) An autosampler (ii) An automated gradient (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room (v) Detector by flame ionization detector (vi) Carrier gas: Nitrogen gas at flow rate of 65ml/min (vii) Temperature: Detector- 201ºC; Injector port- 195ºC; Column- 150ºC (viii) Computer facilities for storing data (ix) Printer for results reporting.

Phenolics (extraction and analysis)

Plant sample (2 g) was weighed into a set of test tubes; 3 ml of 70% acetone in water was added and the tube placed in an ultrasonic water bath at 10ºC for 5 minutes and stirred occasionally with a glass rod. This was filtered through a 50-60µ Gooch crucible into a 50 ml Erlenmeyer flask. The extraction was repeated 3 times by adding 3 ml of 70% acetone in water and allowing it stand in the water bath at 10ºC for 5 minutes. The test tube was rinsed with the final 3 ml portion of 70% acetone in water and emptied into the test tubes. Then 2 ml of 0.1M yb-acetate and 15 ml of 0.1M TEA reagent were added into the filtrate. The flask was closed with rubber stopper, swirled and shaken for 20 minutes after transferring the sample solutions to a set of plastic volumetric tubes, allowed to settle for 4 hours and the supernatant was

collected for analysis using HPLC. The conditions for the analysis of phenolics were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room (v) Detector by fluorescence (vi) Carrier gas: Argon gas at flow rate of 60 ml/min (vii) Temperature: Detector- 120^{o}C; Injector port- 155^{o}C and Column- 117^{o}C (viii) Computer facilities for storing data (ix) Printer for results reporting.

Micromorphological studies of *B. pinnatum*

Epidermal peel: The central portions of the matured leaf were peeled, stained with 1% safranin O and in slides with glycerin. Specimens for anatomical analysis were obtained fresh from matured plants and fixed in formaldehyde-acetic acid alcohol (FAA) for 12 hr. They were transferred to 50% and 70% ethanol and kept at room temperature until required. The petiole, midrib and fruit stalk were hand-sectioned using sharp razor blades (Okoli and Ndukwu, 2002). The sections were stained in 1% Safranin red for two minutes, counter-stained with Alcian blue and mounted on a slide. Thereafter, the slides were viewed and photographed with T340B-LED-5M photomicroscope.

Petiole, stem and root anatomical studies

Specimens for anatomical analysis (petiole, stem and root) were obtained fresh from matured plants and fixed in FAA for 12 hours. They were dehydrated, transferred to 70% ethanol and kept at room temperature until required.

The stem and petiole were hand-sectioned using sharp razor blades (Okoli and Ndukwu, 2002). The sections were stained in 1% safranin O and counter-stained in Alcian blue for two minutes, viewed and micro-photographed using a photomicroscope (T340B-LED-5M) to determine the petiole, stem and root anatomy.

Antifungal Studies of *B. pinnatum*

Pure culture of *Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* were isolated and identified from diseased *Arachis hypogaea* following the isolation method described by Chiejina and Onaebichiemeka (2013) while the fungal cultures identified were confirmed using reference materials (Agrios, 2005; Ellis *et al.*, 2007; Samson *et al.*, 2010).

Aqueous extraction

The aqueous leaf extract of *B. pinnatum* was prepared using the method of Parekh and Chanda (2006) with slight modification by adding the crushed leaf in 100 mL of distilled water. The concentration of each extract was determined by adding 50, 75, 100 and 150 g/mL in different mL of distilled water. The mixture was left for 24 hours at room temperature and afterwards filtered using No. 1 Whatman filter paper. The extract was then concentrated by heating on water bath to 50 mL of the original volume of the extract.

The zone of inhibition:

The zone of inhibition of the extracts was determined using agar well diffusion method by following modified procedure of Ikechi–Nwogu *et al.* (2018). PDA was inoculated with the fungi by spreading the

fungal inoculum on the media. The fungi were later sub-cultured in PDA. Wells were made into the PDA using a sterile 3 mm stainless steel borer. The borer was dipped into alcohol for sterilisation and used to make wells. The wells were then filled up with 0.2 mL of the extracts and care was taken not to allow the solution to spill on the surface of the medium. Different well-isolated colonies of the fungus were selected from a pure agar plate culture, then the top of each colony was touched with a loop, and the growth was transferred to the PDA. Control wells containing sterile distilled water were also plated. The plates were incubated at 28°C for 24 hours and the sensitivity of the organisms to the extract was recorded by measuring the zone of inhibition. This was done by measuring the diameter of the zone of inhibition using a transparent meter rule. The effect of the extracts on the fungal pathogen was compared with that of the water control, respectively.

RESULTS

Proximate Composition

The proximate composition of *B. pinnatum* leaf and root is presented in Table 1. The study showed that the crude fat, ash content, crude fibre, moisture content and protein content were higher in the root (3.70 – 17.65%) than in the leaf (1.55 – 10.42%) while the reverse was the case for carbohydrate (47.49%, 69.64%), in that order. The compositions in the roots were almost twice that in the leaves except for carbohydrate content.

Table 1: Proximate composition of *B pinnatum* leaf and root

Proximate composition (%)	Leaf	Root
Protein content	10.42	17.65
Moisture content	9.41	12.04
Ash content	4.68	8.44
Crude fibre	4.30	10.68
Crude fat	1.55	3.70
Carbohydrate	69.64	47.49

Vitamins

Bryophyllum pinnatum leaves and roots contain both fat-soluble and water-soluble vitamins (Table 2). Water-soluble vitamins (19.45%; 48.22%) were higher than the fat-soluble vitamins (11.75%; 29.05%) in leaves and roots of *B. pinnatum*, respectively. The roots of *B. pinnatum* had higher water-soluble and fat-soluble vitamins than the leaves. The most abundant vitamins in the leaves and roots were vitamin A and vitamin B_9 with percentage occurrence of 98.81% and 99.27% (fat-soluble vitamins) and 83.65% and 83.80% (water-soluble vitamins), in that order.

Table 2: Vitamin content of *B. pinnatum* leaf and root

Class of Vitamins	Vitamin (%)	Leaf	Root
Fat-soluble	A	11.61	28.84
	E	0.07	0.18
	K	0.07	0.03
Water-soluble	B_1	0.05	0.11
	B_2	0.13	0.30
	B_3	0.36	0.86
	B_6	0.17	0.42
	B_9	16.27	40.41
	B_{12}	0.53	1.31
	C	1.94	4.81

Amino Acids

Twenty amino acids were assessed and quantified in the leaves and roots of *B. pinnatum* (Table 3). The essential amino acids (EAA) and non-essential acids (NEAA) of *B. pinnatum* were 5.324% and 1.189% for the leaves and 8.318% and 2.355% for the roots, in that order. The most abundant amino acid was threonine with 58.50% (leaves) and 55.15% (roots) content to the total amino acids (TAA), followed by glycine with 7.60% and 10.86%, respectively. The percentage of EAA and NEAA with respect to TAA present in *B. pinnatum* leaves was 81.74%, 18.26% and 77.93%, 22.07% for leaves and roots, respectively. The TAA was more in the roots of *B. pinnatum* than in the leaves. Also, EAA was more in the leaves and roots than NEAA. However, NEAA was higher in the roots (22.07%) than in the leaves (18.26%) of *B. pinnatum* while the leaves (81.74%) had higher EAA than the roots (77.93%).

Table 3: Amino acids and concentrations in *B. pinnatum* leaf and root

Class of Amino acids	Amino acid (%)	Leaf	Root
Essential	Histidine	0.008	0.008
	Isoleucine	0.004	0.005
	Leucine	0.944	1.782
	Lysine	0.024	0.026
	Methionine	0.433	0.474
	Phenylalanine	0.006	0.007
	Threonine	3.810	5.886
	Tryptophan	0.043	0.073
	Valine	0.052	0.057
Non-Essential	Alanine	0.283	0.309
	Argine	0.021	0.023
	Asparagine	0.005	0.009
	Aspartic acid	0.011	0.012
	Cystine	0.230	0.620
	Glutamic acid	0.053	0.093
	Glutamine	0.010	0.015
	Glycine	0.495	1.159
	Proline	0.008	0.016
	Serine	0.052	0.076
	Tyrosine	0.021	0.023

Phytochemicals

The concentrations of some phytochemicals (phytate, tannin, oxalate, saponin, trypsin-inhibitor, hydrogen cyanide) (HCN)) in the leaves and roots of *B. pinnatum* are presented in Table 4. The roots had a higher concentration of phytochemicals than the leaves; the concentration in the roots was twice that found in the leaves.

The leaves and roots of *B. pinnatum* contained different classes of flavonoids (Table 5). The concentration of total flavonoids in the roots (12.993 g/100 g) of *B. pinnatum* was higher than in the leaves (5.348 g/100 g). The most concentrated flavonoids in the *B. pinnatum* leaves (1.250 g/100 g) was epicatechin while tangeretin was predominant in the root (2.181 g/100 g). Also, the most abundant class of flavonoids in *B. pinnatum* leaves (1.970 g/100 g) and roots (4.078 g/100 g) was flavones. However, flavanones had more individual flavonoids than the other classes of flavonoids.

The leaves and roots of *B. pinnatum* contained different classes of alkaloids (Table 6). The concentration of total alkaloids in the roots (17.80 g/100 g) of *B. pinnatum* was higher than in the leaves (9.63 g/100 g). The most concentrated alkaloid in the *B. pinnatum* leaves (1.786 g/100 g) was caffeine while pyridine was predominant in the root (2.857 g/100vg). Also, the most abundant class of alkaloids in *B. pinnatum* leaves (2.500 g/100 g) and roots (4.478 g/100 g) was pyridine. However, the isoquinoline alkaloids had more individual alkaloids than the other classes of alkaloids.

The phenolic compounds present in the leaves and roots of *B. pinnatum* are presented in Table 7. These phenolics varied in concentration in both leaves and roots. Also, the concentration of phenolic compounds in the leaves (7.437 g/100 g) of *B. pinnatum* was lower than that of the roots (11.622 g/100 g). Cinnamic acid was the most concentrated phenolic in *B. pinnatum* leaves (1.601 g/100 g) and roots (2.232 g/100 g) while the least were singlic acid (0.002 g/100 g) and caffeic acid (0.004 g/100 g) in the leaves and roots, respectively.

Table 4: Phytochemical concentration in the leaf and root of *B. pinnatum*

Phytochemical	Leaf	Root
Phytate (%)	3.870	9.613
Tannin (%)	6.110	15.178
Oxalate (%)	4.883	12.129
Trypsin-inhibitor (%)	1.968	4.888
Saponin (%)	3.425	8.509
HCN (ppm)	0.007	0.018

Table 5: Concentration of flavonoids found in the leaf and root of *B. pinnatum*

Class of flavonoids	Flavonoids (g/100 g)	Leaf	Root
Anthocyanidine	Anthocyanine	0.006	0.016
Flavan-3-ols/flavanols	Catechin	0.020	0.095
	Epicatechin	1.250	1.882
	Epicatechin gallate	0.199	1.007
	Epigallocatechin	0.054	0.055
	Epigallocatechin gallate	0.014	0.048
	Proanthocyanidins	0.019	0.035
	Taxifolin	0.017	0.028
	Theaflavins	0.009	0.017
	Thearubigins	0.011	0.020

Flavanone	Didymin	0.211	0.209
	Eriocitrion	0.008	0.018
	Eriodictyol	0.090	0.880
	Hesperetin	0.119	0.221
	Hesperidine	0.012	0.024
	Nanirutine	0.210	0.208
	Naringin	0.009	0.019
	Naringinenin	0.092	1.900
	Neoeriocitin	0.192	0.215
	Poncirin	0.012	0.021
	Raxifolin	0.011	0.012
Flavones	Acacetin	0.007	0.008
	Apigenin	0.108	0.156
	Diosmin	0.020	0.023
	Luteolin	0.016	0.035
	Neodiosmin	0.678	1.645
	Nobiletin	0.012	0.016
	Rhoifolin	0.010	0.014
	Tangeretin	1.119	2.181
Flavonols	Isorharmnetic	0.004	0.014
	Kaempferol	0.024	0.043
	Myricetrin	0.010	0.013
	Quercetin	0.025	0.048
	Fisetin	0.065	0.157
	Rhamnazin	0.049	0.050
Isoflavones	Diadzein	0.586	1.574
	Genistein	0.021	0.030
	Glycitein	0.002	0.018
	Sinerisetrin	0.027	0.038

Table 6: Concentration of alkaloids found in the leaf and root of *B. pinnatum*

Class of alkaloids	Alkaloids (g/100 g)	Leaf	Root
Acridine	Acridine	0.009	0.010
Colchicine	Colchicine	0.021	0.061
Imidazole	pilocarpine	0.933	1.801
Indole/benzypyrole	β-carboline	0.020	0.049
	Ergotamine	0.909	1.993
	Eserine	0.051	0.094
	Rauwolfia	0.052	0.060
	Reserpine	0.005	0.014
	Strychnine	0.009	0.023
Isoquinoline	Berberine	0.016	0.027
	Cephaline	0.006	0.007
	Codeine	0.624	0.727
	Emetine	0.009	0.016
	heroine	0.002	0.021
	Morphine	0.955	1.113
	Nacortine	0.017	0.020
	papaverine	0.025	0.029
	Psychotrine	0.259	0.648
	Tubocurarine	0.112	0.296
	Apomorphine	0.014	0.016
Piperidine	Conine	0.006	0.009
	Lobeline	0.095	0.394
	Piperine	0.021	0.045
Purine (Pseudo)	Caffeine	1.786	2.081
	Theobromine	0.025	0.026
	Theophylline	0.018	0.099
Pyridine	Nornicotine	0.015	0.044
	Pyridine	1.700	2.857
	Ricinine	0.745	1.497
	Nicotine	0.019	0.022
	Peletrevine	0.021	0.058
Quinolone	Cinchonidine	0.091	0.319
	Cinchonine	0.098	1.091
	Quinidine	0.006	0.015
	Quinine	0.047	0.055
	Quinoline	0.004	0.012
Tropane	Apoatropine	0.115	0.134
	Atropine	0.047	0.055
	Hyoscine	0.025	0.049

	Cocaine	0.049	0.054
Vinca	Vinblastine	0.016	0.034
	Vincristine	0.010	0.018
β-Phenylethylamine	Ephedrine	0.013	0.115
	Norpseudoephedrine	0.013	0.017
	Phenylethylamine	0.597	1.675

Table 7: Concentration of phenolics found in the leaf and root of *B. pinnatum*

Phenolics (g/100 g)	Leaf	Root
Aesculetin acid	0.015	0.028
Astringin acid	1.010	2.012
Benzoic acid	0.019	0.026
Cafein acid	0.010	0.014
Caffaric acid	0.006	0.013
Caffeic acid	0.005	0.004
Carreic acid	0.013	0.143
Castarinol C1 acid	0.590	0.684
Castarinol C2 acid	0.023	0.098
Castarinol C3 acid	0.029	0.034
Castarinol C4 acid	0.071	0.082
Catechin acid	0.139	0.245
Cinnamic acid	1.601	2.232
Contaric acid	0.006	0.013
Coumaric acid	1.104	1.279
Cutissin acid	0.029	0.034
Cyanidin 30-glycoside	0.006	0.007
Cyanidin Coumaryl 30 glycoside	0.032	0.037
Ethyl/Caffeati acid	0.012	0.014
Ethyl/gallon acid	0.004	0.007
Ferteric acid	0.013	0.027
Ferulic acid	0.015	0.021
Galic acid	0.012	0.017
Genticitic acid	0.856	1.194
Homogentisic acid	0.559	0.780
Homovalinic acid	0.022	0.031
Homovalnillic acid	0.011	0.018
Izoferulic acid	0.639	1.104
Mendelic acid	0.007	0.016
M-OH-Benzoic acid	0.274	0.590

P-cumaric acid	0.029	0.071
Piperonic acid	0.009	0.012
P-OH-Benzoic acid	0.005	0.012
P-OH-Phenyloacetic acid	0.014	0.032
Protocatechic acid	0.014	0.029
Pyrogallic acid	0.017	0.024
Sacitic acid	0.008	0.006
Salicylic acid	0.042	0.059
Sinagic acid	0.048	0.385
Sinamic acid	0.042	0.059
Singlic acid	0.002	0.015
Syringic acid	0.006	0.008
Tannic acid	0.014	0.029
Valnilic acid	0.046	0.065
Veratoc acid	0.009	0.012
Total Phenolics	7.437	11.622

Mineral Composition

The minerals of *B. pinnatum* assessed and their concentrations are presented in Table 8. The concentrations of these minerals were higher in the roots than in the leaves.

Table 8: Mineral composition of the leaf and root extracts of *B. pinnatum*

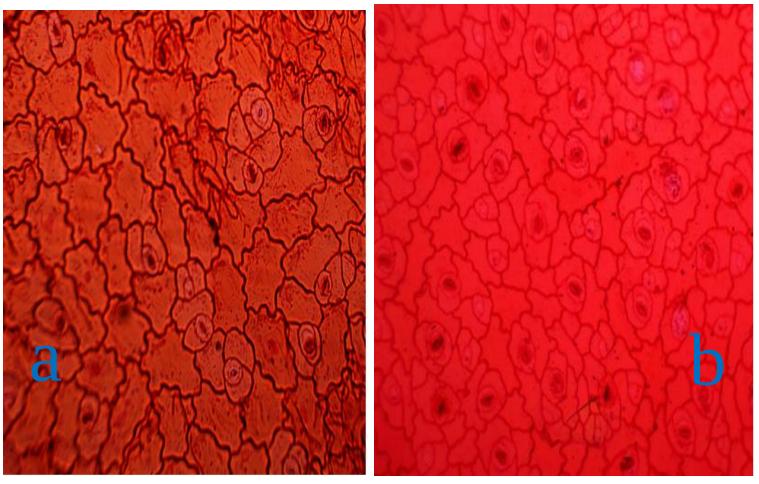
Mineral	Leaf	Root
Calcium (%)	1.874	3.375
Magnesium (%)	0.551	0.993
Potassium (%)	1.041	1.875
Sodium (ppm)	0.0000214	0.000214
Manganese (ppm)	0.0089008	0.0160307
Iron (ppm)	0.0056213	0.0101247
Zinc (ppm)	0.0037475	0.0067498
Copper (ppm)	0.0012648	0.0022781

Micromorphology Study

The foliar epidermis of *B. pinnatum* revealed anisocytic stomatal type, that is, the stomata have guard cells between two larger subsidiary cells and distinctly smaller one. This observation can be seen in the upper and lower surfaces of the leaves (Plates 1a & 1b).

The transverse section (TS) of *B. pinnatum* stem indicates that it is a dicot plant (Plate 2). The vascular bundles in the stem were arranged in concentric layers of circle. The stem of the plant revealed the presence of tannins as indicated by the black spots found in the vascular bundle, secretory cells and

the cortex. The cells of the petiole of *B. pinnatum* also contained tannins (Plate 3). The black spots close to the epidermis, within the cortex and vascular bundles indicated the presence of tannins. The TS of the root revealed the epidermis, secretory cells and vascular bundles which are parenchymatous, the pith and the presence of starch grains (Plate 4).



Plates 1(a & b): *B. pinnatum* leaves (a) Upper epidermis (b) Lower epidermis

Plate 2: TS of *B. pinnatum* stem

Plate 3: TS of *B. pinnatum* petiole

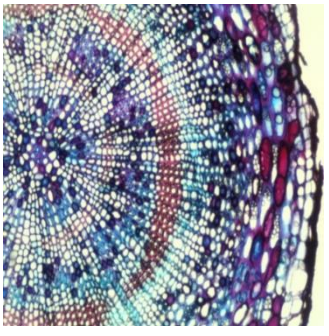


Plate 4: TS of *B. pinnatum* root

Antifungal activities of *B. pinnatum* leaves

The antifungal activities of *B. pinnatum* leaf extract against some phytopathogenic fungi (*A. niger, A. flavus, R. stolonifer*) of *Arachis hypogaea* revealed different responses to the leaf extract. Mycelial growth of these fungi and their percentage inhibition by the active ingredient in the *B. pinnatum* leaf extract are presented in Figures 1 and 2, respectively. The mycelial growth of these fungi ranged from 2.57 – 3.33 cm which was lower when compared with the control treatment (8.7 cm) while the percentage inhibition

ranged from 61.60 – 70.50%. The *B. pinnatum* leaf extract inhibited the *R. stolonifer* mycelial growth more, followed by *A. flavus* and *A. niger* in that order, while the reverse was the case for percentage inhibition.

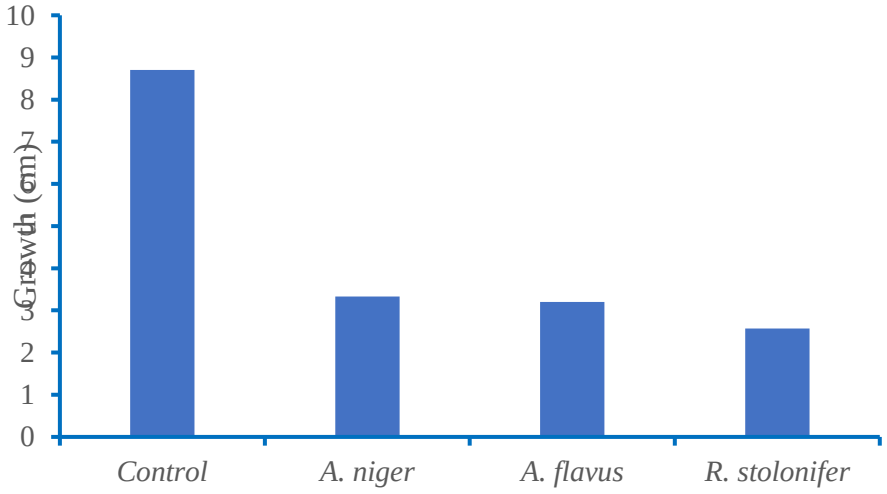


Figure 1: Mycelial growth of fungi in aqueous extract of *B. pinnatum*

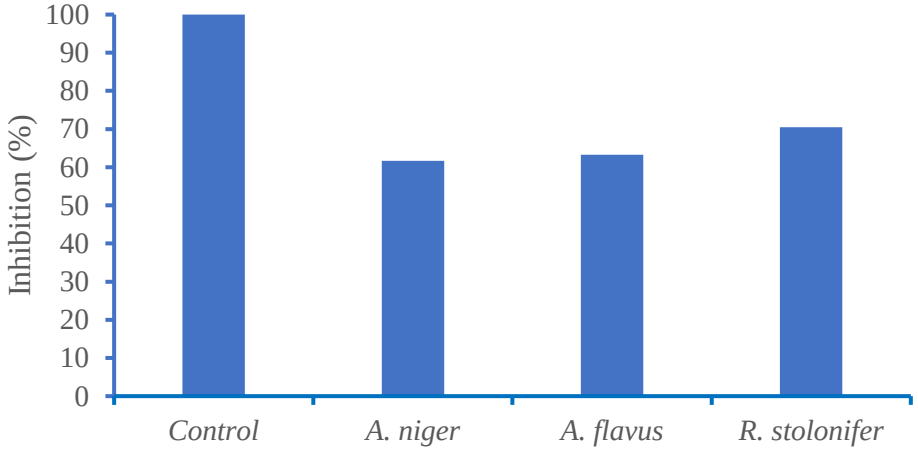


Figure 2: Percentage inhibition of *B. pinnatum* extract on fungal growth

DISCUSSION

Results of the proximate composition of *B. pinnatum* showed appreciable levels of carbohydrate, protein, fats, oil, crude fiber, ash and moisture contents in the leaves and roots. Carbohydrate content was highest in the leaves (69.64%) and roots (47.49%) while crude fat was lowest. This result differed from the findings of Nwali *et al.* (2014) who reported high values of carbohydrate of 72.92±1.08% in *B. pinnatum* leaves. The result is within the range 51.17 – 66.29% for carbohydrate content of *T. tetraptera* as reported by Uyoh *et al.* (2013). This level of carbohydrate provides energy to the cells in the body, especially the brain, which solely depends on carbohydrate (Effiong *et al.*, 2009). The higher moisture content recorded in the roots of *B. pinnatum* than in the leaves indicates that the roots have increased microbial activities during storage. This finding is in line with Abdullahi (2002). The moisture contents in the leaves (9.41%) and roots (12.04%) were higher than those recorded by Wasagu *et al.* (2013) on *P. stratiotes* leaves (4.50%) and roots (4.50%).

Vitamin A in *B. pinnatum* was higher compared with 0.48 % and 1.5 % for leaves and roots, respectively reported in *P. stratiotes* by Wasagu *et al.* (2013). Vitamins C and E were lower in this study than earlier report except for vitamin C in *B. pinnatum* roots. The high value recorded for vitamin A in *B. pinnatum* leaves and roots indicates that it is important for normal vision, immune system and for proper functioning of the heart and lungs. George (2009) reported that vitamin E provides protection, including its function as an antioxidant and anti-inflammatory processes, inhibition of platelet aggregation and immune enhancement. Bjelakoni *et al.* (2007) reported that vitamin C blocked many debilitating diseases and increased the body's immunity. Vitamin C content of *B. pinnatum* may have been influenced by the nutrient status of the soil. Welch (2002) observed that excessive N fertilizers could adversely affect the accumulation of vitamin C in vegetables and fruits while increased supply of K fertilizer to the crops resulted in increased vitamin C content by about 8 – 20% depending on the species. Vitamin B_{9i} is the most abundant water-soluble vitamin that can help the body convert carbohydrates into glucose to produce energy.

The total amino acids in *B. pinnatum* leaves and roots were lower than the range 59.58 – 72.24% reported by Inyang (2016) for *Heinsi acrinita, L. africana, C. esculenta* and *I. batatas.* The presence of these amino acids in the body helps to promote cell immune defense function.

The tannin content of *B. pinnatum* leaves and roots was higher than the range reported by Uyoh *et al.* (2013) for *T. tetraptera* fruits. According to Chikezie *et al.* (2008), tannins are considered as dietary anti-nutrients responsible for the astringent taste and poor palatability of foods and drinks. Hydrogen cyanide content was lower compared to other phytochemicals. This low concentration explained why *B. pinnatum* can be used in the raw form for treatment of ailment. The phenolics are known for their anti-inflammatory, 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, 2007). 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*., 2012).

Calcium was found to be the most abundant macro-element present in the *B. pinnatum* in both leaves (1.874%) and roots (3.375%). Normal extracellular calcium concentration is necessary for blood coagulation and for the integrity, intracellular cement substance. It also helps in the development of strong bone and teeth. The presence of zinc in the plant could mean that the plant can play valuable roles in the management of diabetes which results from insulin malfunction (Dauda and Garba, 2017). Trease and Evans (1989) 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 (2002), 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, 2000; White and Broadley, 2009).

Tannins have astringent properties which hasten the healing of wounds. The micro-morphological studies show that the upper and lower epidermis contains anioscytic stomata types in which the stoma is completely surrounded by three subsidiary cells, variable in position and shape. This is in line with the report of Prabhakar (2004) on classification of stomata.

The observation on the antifungal effect of aqueous leaves extract of *B. pinnatum* on *A. niger, A. flavus* and *R. stolonifer* is in line with the work of Thippeswamy *et al.* (2014), who reported the biosynthesis of aflatoxin by *A. flavus* using aqueous and solvent extracts of *B. pinnatum* and other plant extracts, which showed a high degree of antifungal properties and aflatoxin inhibition. Nwadinigwe (2011) reported that aqueous extracts of *B. pinnatum* is less effective on the inhibition of *A. niger* as compared with methanol extracts.

CONCLUSION

The study revealed that *B. pinnatum* contains appreciable levels of carbohydrates, proteins, fat, oil, crude fiber, ash, moisture and mineral contents, essential amino acids (EAA) and non-essential acids (NEAA), phytate, tannin, oxalate, saponin, trypsin-inhibitor, hydrogen cyanide, different subgroups/classes of flavonoids, alkaloids, phenolic compounds in the leaves and roots. The presence of these phytochemicals, proximate and mineral elements in this plant could be the reason the plant is recommended for various healing claims. This also shows *that B. pinnatum* is a good source of nutrition for humans and should be incorporated in our daily diet. *Bryopyllum pinnatum* is an intrusive plant which spreads and occupies any available space. The antifungal activities observed in this study indicate the potential for the use of the weed as a raw material for the production of fungicides that can be used in the control of plant diseases caused by mycelial growth of *R. stolonifer*, *A. flavus* and *A. niger*, respectively. The inhibitory effects of *B. pinnatum* extracts on these fungi indicate its phytochemical constituents. This method of plant disease control is viable economically and poses little or no environmental hazard. Furthermore, the plants are accessible to farmers who may not have access to artificial fungicides.

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