Innovations

Phytochemicals, Mineral Compositions and Antibacterial Susceptibilities of Leaf-Extracts of Bryophyllum Pinnatum

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Abstract: *The study investigated the mineral, heavy metal, phytochemical compositions and antibacterial sensitivities of Bryophyllum pinnatum leaf-extracts against bacterial pathogens. The phytochemicals were extracted with absolute ethanol, methanol and aqueous solution as solvents. The phytochemical and mineral compositions of the extracts were determined using Association of Analytical Chemist (AOAC) and Atomic Absorption Spectrophotometeric (AAS) methods respectively. Antibacterial sensitivity tests were conducted using agar well diffusion assay method against some pathogenic bacteria, including Staphylococcus aureus, Proteus mirabilis, Streptococcus pyogenes, Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli. The susceptibility patterns of these extracts against these pathogens were compared with those of standard antibiotic (Chloramphenicol), which served as a positive control. Minerals and heavy metals such as K,Na, Ca, Mg, Pb, Mn, Zn, Cu, Hg, Cd, Ni, and Co were detected in low amount in the extracts. Secondary metabolites like alkaloids, flavonoids, tannins, saponins, and phenolic compounds, were identified in the extracts. Aqueous extract was the most effective, with a mean inhibition range of 0.00-12.64±0.01 mm, followed by ethanol (0.00-12.25±0.21 mm), while methanolic extract was the least (0.00-11.00±0.00 mm). Streptococcus pyogenes was the most susceptible bacterium, while Proteus mirabilis was the least. The bacteria were however more susceptible to Chloramphenicol than the three extracts at the tested concentrations. This study provides valuable insight into the potential use of Bryopyllum pinnatum as an antimicrobial agent.*

Keywords: *Heavy metals, phytochemicals, ethanol, methanol, aqueous, extracts*

1.0 Introduction

Plants are natural gifts that can be found all around us. Although some plants are prominent in certain places, they can be transplanted to a new habitat provided the environmental conditions are favorable. Many plants parts are not only sources of food, but also serve as herbs and thus medicinal. Researches on various parts of plants have established them as sources of antimicrobial agents [1]. Plants have been used as a source of medicine since the beginning of time, and these plants are known as "medicinal plants." Due to its diversity, the kingdom contains a wide range of plants that can help treat a variety of ailments and diseases. Many of these plants have been discovered over time, but there are still many more to find [2].

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant [3]. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [4].

Substances that kill bacteria are commonly referred as antibiotics. They can be used on the skin, taken intravenously or orally. They can be in liquid or powdered forms. Extensive use of antibiotics has resulted in emergence and re-emergence of antibiotic resistance amongst bacteria, which has become a public health issue worldwide and has been documented. This emergence of antibiotic resistance has also been linked with the wrong use of antibiotics. Many people in impoverished and underdeveloped nations are unable to afford adequate medical care and as a result, rely on patent medication stores when they are ill [5]. This has resulted in ongoing medication addiction, particularly of antibiotics, as wide range medicines are frequently prescribed and abused. When the proper medications are provided, most people either do not finish the specified dosage by the doctor, especially when their health improves or do not take it at all. As a result, there is a need to research novel and safer antimicrobials that will likely operate differently from those now in the market [6].

Bryophyllum pinnatum is an environmental weed from the family Crassulceae and the common names include life plant, love plant, miracle leaf, and Cant1erbury bells [7]. It is widely distributed in tropical Africa, America, Hawaii, India, China, Australia, and Madagascar [8]. In ethno-medicine, Bryophyllum pinnatum is used to cure ear-aches, coughs, diarrhea, dysentery, abscesses, ulcers, insect bites, heart problems, epilepsy, arthritis, dysmenorrhea, and whitlow [9]. In literature, much work has been evaluated the phytochemicals and antimicrobial sensitivities of Bryophyllum pinnatum plant. However, there is paucity of information on the mineral and heavy metal contents of this plant in literature.

This study therefore aims to evaluate the mineral and heavy metal contents, phytochemical composition, as well as the antibacterial potential of the plant leaf-extracts against some bacterial pathogens.

2.0 Materials and Methods

2.1.Collection of Plant Materials

Bryophyllumpinnatum leaves were sourced from the botanical garden in the Department of Biology, School of Biological Sciences, Federal University of Technology, Owerri. The plant materials were air dried at room temperature to obtain constant weight (loss of moisture), and thereafter, pulverized into powder, using a mechanical grinder, previously washed with detergent and rinsed severally with copious amount of water. The leaf-powder was then stored in an air-tight container.

2.1.2 Collection of test microorganisms for antimicrobial activity

Bacterial cultures of Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Proteus mirabelis, Pseudomonas aeruginosa, Escherichia coli, were collected from the Microbiology Laboratory, Federal Medical Center (FMC), Owerri, Imo State. They were sub-cultured in a Nutrient Agar (NA) plates to ascertain their purity state and thereafter, preserved in the refrigerator, on agal slant until needed.

2.2 Plant Extraction

The extraction method used in the study was cold maceration extraction method as described [10] with modification which involves simple soaking, filtering and evaporations. Three solvents were used in the extraction, which were absolute ethanol, methanol and aqueous solution. Thirty (30g) grams of the powdered sample was weighed into three conical flasks and 150 ml of ethanol was added into one of the flasks. Similarly, 150 ml each of methanol and aqueous solution were respectively added into the other two conical flasks. The contents of each flask were properly mixed and corked tightly, allowed to stand for 24 hours and thereafter, filtered with Whatman filter paper. The filtrate was evaporated in a water-bath to obtain a jelly like extract.

2.3 Phytochemical Analyses

Phytochemicals were analyzed using qualitative and quantitative chemical techniques.

2.3.1 Qualitative analyses

These analyses were carried out to establish the presence or otherwise of the different bioactive compounds in the extracts, using the methods of [11, 12].

Test for tannins

One (1.0 ml) milliliter of 1% concentrated HCl was pipetted into a 250 ml conical flask and made up to 100 ml with distilled water. Then, three (3.0g) grams of the extract was added to 2.0 ml of the 1% HCl. The presence of red colour or precipitate indicates the presence of phlobotannins.

Test for saponins

Two (2g) grams of the extract was boiled in 20 ml of distilled water in a water bath and filtered. Ten (10 ml) milliliters of the filtrate were mixed with 5 ml of distilled water and shook vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, followed by vigorous shaking, and thereafter, observed for the formation of emulsion.

Test for alkaloids

Five ml (5ml) of the extract was added to 2 ml of HCL. To this acidic medium, 1 ml of Dregendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Ferric chloride test for phenolic nucleus

One (1.0 ml) milliliter of the extract was pipetted into a 15 ml test tube, and then 1.0 ml of 10% Ferric chloride was also pipetted into the test tube, mixed and observed for any change in colour. Greenish brown or black colour/precipitate is an indication of presence of phenolic nucleus.

Test for flavonoids

One (1ml) milliliter of the extract was pipetted into a 15 ml test tube, followed by 1.0 ml of 10% lead acetate solution in the same test tube. These were mixed properly and observed for colour change or precipitate which indicates presence of flavonoids

Test for cardiac glycosides

One (1ml) of the extract was added 10 cm^3 of 50% H_2SO_4 and was heated in boiling water for five minutes. Ten (10 cm^3) cubic-centimeter of Fehling's solution (5cm^3 each of solutions A and B) was added and boiled. A brick red precipitate indicating presence of glycosides was observed.

Test for terpenoids

Five (5ml) milliliter of each extract was mixed in 2ml of chloroform (Numex, India) and then concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inner face was formed to show positive results for the presence of terpenoids.

Test for glycosides

Ten (10g) grams of the extract were hydrolyzed with HCL for few hours on a water bath. Then, 1ml of pyridine was added to the hydrolysate and few drops of sodium nitroprusside solution were also added and the mixture made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

2.3.2 Quantitative analyses

Determination of phenol

Ten (10g) grams of the sample was dissolved in 50 ml of hexane and then extracted 3 times with 30 ml each of 80% aqueous methanol. Thereafter, make up the extract to 100 ml and leave to stand overnight. To a 1ml aliquot of the extract, 5 ml folin – ciocalteus phenol reagent was added and shook to mix very well and then left to settle for 5 minutes. One (1 ml) milliliter of Na_2CO_3 added and shook before standing for 1 hour at room temperature. Thereafter, the absorbance was read at 725nm using UV-Vis spectrophotometer.

For the preparation of calibration curve, 1 ml aliquot of 0.05, 0.2, 0.4, 0.5 and 0.6 mpl/1 aqueous gallic acid solution were mixed with 5ml folin-ciocalten reagent and 1 ml Na_2CO_3 solution. The optical density was measured after 1 hour at 756 nm in rapport with a blank solution (1 ml bi-distilled water, 5 ml folin – ciocalten reagent and 1 ml Na_2CO_3 solution).

Curve

Y = 0.0008x - 0.0057

Estimation of saponins

Each extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, and then 2 ml of 72% H_2SO_4 was added, mixed well and heated for 10 min on a water bath at 60°C, absorbance was read at 544nm with a spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan) against reagent blank. Diosgeninis was used as a standard material and the assay was compared with Diosgeninis equivalents.

Determination of steroids

Gravimetric method described [12] was used in determining of the steroid content of the extract. One gram of the extract was weighed into a 250-ml conical flask, followed by the addition of 10 ml of chloroform, and then stirring continuously at room temperature. Thereafter, the mixture was made up to 100 ml with chloroform, filtered with Whatman filter paper No 1. A 3 ml portion of the filtrate was pipetted into a test tube and 2 ml of Liberman-Burchard reagent was added and thoroughly mixed. The absorbance of the mixture was measured using spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 640nm. Standard cholesterol solution

ranging from 0 - 2.5 mg/ml were prepared and also treated with 2 ml each of Liberman-Burchard reagent and their absorbance read using spectrophotometer at the same wavelength (640nm). Finally, the amount of steroid present in the date flesh was measured by extrapolation.

Estimation of alkaloids

To 1ml of the extract, 5 ml each of phosphate buffer (pH 4.7) and BCG solution were added, thereafter, the mixture was shook with 4 ml of chloroform. The extract was collected in a 10-ml volumetric flask and then diluted by adjusting the volume with chloroform. The absorbance of the complex in chloroform was measured spectrophotometerically at 470 nm against the blank prepared as above but without extract. Atropine was used as a standard material and compared the assay with Atropiene equivalents.

Estimation of flavonoids

Total flavonoid content was determined by Aluminum chloride method using catechin as a standard. One milliliter of the extract and 4 ml of water were added to a 250-ml volumetric flask. After 5 min, 0.3 ml each of 5 % Sodium nitrite and 10% Aluminum chloride were added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Determination of tannin

The amount of total tannin in the samples was determined using the methods described [13] with slight modifications. The extract (1g in duplicate) was dissolved in 80 ml of distilled water and boiled for 30 mins. The solution was cooled, transferred into a 100-ml volumetric flask and made up to mark with distilled water. The solution was filtered with Whatman filter paper No 1. Folin Denis reagent and saturated sodium carbonate solution were prepared in accordance with the standard for tannin content analysis. Also standard solution of tannic acid was freshly prepared and aliquots (0ml, 0.5ml, 1ml, 1.5ml, 2ml, and 2.5ml) were dispensed into 25-ml volumetric flasks. 1.25ml of Folin-Denis reagent and 2.5ml of sodium carbonate solution were added to each flask. Each mixture was made up to the volume of the flask with distilled water. The colour was measured after 30 mins using spectrophotometer at 760nm. To a five millimeter (5ml) portion of the filtrate in the volumetric flask was added 1.25ml of Folin-Denis reagent and 2.5ml of Sodium carbonate solution. The colour was measured after 30mins using spectrophotometer at 760nm. The amount of Tannin content was measured by extrapolation.

Cardiac glycosides determination

To 1ml of the extract was added 1ml of 2% solution of 3,5 – DNS (Dinitrosallcyclic acid) in methanol and 1ml of 5% aqueous NaOH. The mixture was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50°C till dryness and weight of the filter paper with residue was noted[14].

The cardiac glycoside was calculated in %

Calculation

$$\% \text{ cardiac glycoside} = \frac{(\text{Weight of filter paper + residue}) - (\text{weight of filter paper})}{\text{Weight of sample analysed}} \times 100$$

Oxalate determination (Titration method)

This was determined according to [14].This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion

Two (2g) grams of sample was suspended in 190 ml of distilled water in a 250-ml volumetric flask, 10ml of 6M HCl ass added and the suspension digested at 100°C for 1 hour, cool, and then made up to 250 ml mark before titration.

Oxalate precipitation

Duplicate portions of 125 ml of the filtrate were measured into beakers and four drop of methyl red indicator added. This was followed by drop wise addition of NH_4OH solution until the test solution changes from salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion was then heated to 90°C, cooled and filtered to remove precipitate containing ferrous ions. The filtrate was again heated to 90°C and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25°C. The solution was then centrifuge at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate titration

At this point, the total filtration resulting from digestion of 2g of sample was made up to 300 ml, aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink colour which

persists for 30 seconds. The calcium oxalate content was calculated using the formula below:

$$\text{Oxalate Content (mg/100g)} = \frac{T \times (Vme)\ (Df) \times 10^5}{ME \times Mf}$$

Where T is the titre of $KMnO_4$(ml), Vme is the volume – mass equivalent (i.e. 1ml of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4) where Vt is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of sample used.

Test for phytate

Phytate contents were determined using the method of [15]. A 0.2g portion of each of the differently processed extracts was weighed into different 250-ml conical flasks. Each extract was soaked in 100 ml of 2% concentrated HCl for 3 hours. The extracts were then filtered. Fifty (50ml) milliliter of each filtrate was placed in 250-ml beaker and 100 ml distilled water added to each sample. Ten (10ml) milliliter of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard Iron (III) chloride solution which contained 0.00195g iron per ml. The percentage phytic acid was calculated using the formula:

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

Estimation of total terpenoid content

The total terpenoid content of the extracts was determined based on an assay described [16] with some modifications. Linalool was used as the standard for estimation. An aliquot of the reaction mixture obtained after Salkowski test was employed for the qualitative analysis of terpenoids in the extract was transferred to colorimetric cuvette. The absorbance was measured at 538 nm against blank (95% (v/v) methanol). For the standard curve, 200 µl of linalool solution in methanol was added with 1.5 ml chloroform and serial dilutions [dilution level-100 mg/200 µl to 1 mg/200µl linalool Conc.] were prepared in which total volume of 200 µl was made up by the addition of 95% (v/v) methanol. Calibration curve of linalool was plotted and the total terpenoid content expressed as milligrams of linalool equivalents per gram of dry weight (mg linalool/g DW) was determined using the regression equation. Extracts were analysed in duplicates.

Hydrogen cyanide determination

About 5g of the powdered extract was dissolved in 50 ml of water in a 100-ml conical flask, corked and was allowed to stay overnight. The solution was then filtered with media paper for cyanide determination. About 1 ml of the filtered solution was transferred into another 100-ml conical flask and 4 ml of alkaline picrate solution was

added and incubated at 50°C in a water bath for 5 mins. Colour development and absorbance was taken at 490 nm. A blank preparation using 1ml distilled water was made. The cyanide content was extrapolated using standard curve and reported as mean of duplicate determination.

2.4. Procedure for Mineral Analysis

The mineral contents of the plant leaf were determined as described [17], [18]. One (1.0 g) gram of powdered sample was weighed into a crucible and then heated to ash in a furnace at 580°C for 3 hrs. The ash was cooled in a desiccator and thereafter 10 ml of conc. HNO_3, 4 ml of $HClO_4$ and 1ml of H_2SO_4 were added and digested at high temperature till a clear content was obtained. The tube was then cooled and the solution transferred quantitatively to 100-ml volumetric flask and the final volume adjusted to 50 ml using distilled water. This solution was then used for the determination of the mineral contents using atomic absorption spectrometry.

2.5. Antimicrobial Analyses

2.5.1. Preparation of 0.5 McFarland standard solution

One gram of $BaCl_2$ was weighed and added to 99 ml of sterilize water. This gives 1% $BaCl_2$ solution. One (1%) percent v/v H_2SO_4 was also prepared by diluting 1ml of concentrated H_2SO_4 in 99ml of sterilize water. An aliquot (0.05ml) of 1% v/v $BaCl_2$ was added to 9.90 ml of 1% v/v, H_2SO_4 to a 15-ml test tube, the suspension gives turbidity equivalent to $1.0x10^8$cfu/ml, which is a standard inoculum.

2.5.2. Preparation of 24 hours bacterial inoculum

Nutrient agar medium were prepared according to the manufacture's instruction and dispensed in petri-dishes. These plates were then inoculated with the pure culture of the different bacterial isolates earlier persevered in the refrigerator, using streak plate method. The plates were thereafter incubated in an incubator at 37°C for 24 hours. These twenty-four hours fresh cultures were used in preparing the standardized inoculum for the sensitivity test [19].

2.5.3. Standardization of bacterial inocula

To prepare and standardize the inocula, from the 24 hours old culture of bacteria isolates in petri-dishes, a loop full of each isolate was transferred into 5 ml of normal saline in four different 10 ml test tubes (0.9%/v) and allowed to stand for 10 minutes, then vortex for 1 minute at 50 rpm. Then the turbidity of each bacterial isolate in normal saline was matched against that of 0.5 McFarland standard solutions in a test tube, through adjusting and reading out the absorbance at the same wavelength as the standard. Once the turbidity of the two suspensions equals, it gives a bacterial suspension equivalent to $1.0x10^8$cfu/ml.

2.5.4. Preparation of culture media/reagents

All media and reagents used were prepared according to the manufacturer's guide

2.5.5. Sensitivity test

Susceptibilities of the test isolates to Bryophyllum pinnatum leaf extracts were done using agar well diffusion assay techniques as described [20]. Three wells of 6.25mm were made with a sterile cork borer on Nutrient agar plates (in duplicates), previously seeded with 24 hours old cultures of the test bacteria (Staphylococcus aureus, Proteus mirabilis, Streptococcus pyogenes, Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli). The wells were filled with different concentrations (25%, 50% and 100%) of the ethanol, methanol and aqueous extracts of the plant leaf and the plates incubated for 24 hours at 37°C. Thereafter, the zones of inhibition around the wells were measured with meter-rule and recorded in millimeters (mm). Chloramphenicol antibiotic at the same concentrations were used as positive control.

2.5.6. Minimum Inhibitory Concentration (MIC) Assay

The Minimum Inhibitory Concentration Assay is a technique used to determine the lowest concentration of a particular antibiotic or plant extract needed to kill an organism. The MIC of the extracts was calculated by diluting the extracts twice (starting at 40 mg/ml) with nutritional broth in a series of test tubes, and then adding an equal volume of the test bacterium to each tube, and incubating the tubes at 37°C for 24 hours. Tubes were inoculated with cell suspensions instead of extracts to create controls. After the incubation period, the tubes were checked for the existence of turbidity. The minimum inhibitory concentration will be the lowest concentration at which there was no discernible bacterial growth in comparison to the control (MIC).

2.6. Data Analysis

The analyses were carried out in duplicates. The mean values were then calculated using Microsoft Excel package, 2010 and the values reported as the mean ± standard deviation.

3.0 RESULTS

3.1 Qualitative Analysis of the Extracts

The qualitative analysis of the leaf extracts are shown in Table 1. From the results, flavonoids were moderately extracted in both alcoholic extracts while highly extracted in aqueous extract. Similar results were obtained for alkaloids. Saponins, phenols, hydrogen cyanide and phytates showed low extraction across the three extracts. Cardiac glycosides and tannins were only present in low amount in methanolic and aqueous extracts respectively, while absent in the other extracts.

Table 1: Qualitative Analysis of B. pinnatum Leaf Extracts

Parameters	Extracts		
	Ethanol	Methanol	Aqueous
Flavonoids	++	++	+++
Saponins	+	+	+
Terpenoids	+	++	+
Phenols	+	+	+
Alkaloids	++	++	++
Cardiac Glycosides	-	+	-
Tannins	-	-	+
Steroids	++	+	+
Hydrogen Cyanides	+	+	+
Oxalates	+	+	++
Phytates	+	+	+
+++ = High, ++ = Moderate, + = Low, - = Not detected			

3.2. Quantitative Analysis of the Extracts

The quantitative phytochemical analysis result of the extracts showed that aqueous solvent extracted the highest amount of flavonoids (411.77 ± 4.17 μg/g), followed by ethanol (197.06 ± 4.16 μg/g), while methanol had the least (76.56 ± 0.13 μg/g) (Table 2). Similar trends were observed among the extracts for terpenoids, cardiac glycosides and steroids. Tannins and oxalates also recorded highest extraction in aqueous solvent, with mean values of 63.09 ± 0.06%, and 1181.25 ± 79.55 mg/100g respectively, followed by methanol (46.44 ± 0.08%, 281.25 ± 79.55 mg/100g), while ethanol extracted the least amount of the phytochemicals (34.75 ± 0.09, 112.5 ± 0.00 mg/100g). More saponins were extracted in methanol (742 ± 22.49 μg/g) and ethanol (724.21 ± 5.96 μg/g) than in aqueous solvent (102.04 ± 2.96 μg/g). Phenols and phytates concentrations in all the extracts were however low.

Table 2: Quantitative Analysis of B. pinnatum Leaf Extracts			
Parameter	Extracts		
	Ethanol	Methanol	Aqueous
Flavonoids(μg/g)	197.06 ± 4.16	76.56 ± 0.13	411.77 ± 4.17
Saponins (μg/g)	724.21 ± 5.96	742 ± 22.49	102.04 ± 2.96
Terpenoids (mg/100g)	0.28 ± 0.06	0.20 ± 0.06	0.48 ± 0.11
Phenols (mg/g)	0.01 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Alkaloids (μg/g)	0.69 ± 0.08	4.85 ± 0.04	3.01 ± 0.04
Cardiac Glycosides (%)	14.61 ± 0.31	14.60 ± 0.77	18.33 ± 0.04
Tannins (%)	34.75 ± 0.09	46.44 ± 0.08	63.09 ± 0.06
Steroids (mg/100g)	10.32 ± 0.04	1.92 ± 0.07	11.42 ± 0.06
Hydrogen Cyanides (mg/g)	26.56 ± 0.01	46.95 ± 0.10	22.10± 0.021
Oxalates (mg/100g)	112.5 ± 0.00	281.25 ± 79.55	1181.25 ± 79.55
Phytates (%)	0.06 ± 0.00	0.05 ± 0.00	0.03 ± 0.00
Value = Mean ± SD			

3.3. Susceptibility Pattern of the Test Bacteria Against the Extracts

The susceptibility pattern of the test bacteria against the extracts at different concentrations is shown in Table 3. The susceptibility pattern of the test bacteria was directly proportional to the concentrations of the extracts. Against ethanolic extract for example, at 25% concentration, S. aureus, Salmonella typhi, Proteus mirabilis, Escherichia coli, showed no zones of inhibition. Similar result was also observed at the same concentration for methanolic extract against the other test bacteria except Staphylococcus aureus and Streptococcus pyogenes. Streptococcus pyogenes was the most sensitive bacterium at higher concentrations of the ethanolic extract, recording 9.33±0.11, and 12.25±0.21 mm zones of inhibition at 50 and 100% concentrations respectively.

In methanolic extract, at 100% concentration Streptococcus pyogenes was the most sensitive (11.00±0.00 mm), followed by Staphylococcus aureus (10.75±0.02 mm), with Proteus mirabilis being the least inhibited (7.39±0.01 mm).

At 25% concentration of the aqueous extract, Staphylococcus aureus, and Proteus mirabilis showed no zone of inhibition, while Pseudomonas aeruginosa was the most susceptible of the test organisms at 50% concentration (11.22 ± 0.12 mm). Similar result was observed at 100% concentration of the same extract. Generally, the bacteria were more susceptible to Chloramphenicol than the three extracts at the tested concentrations.

Table 3: Susceptibility Pattern of the Test Organisms Against the Extracts

		Mean Zones of Inhibition (mm)					
Extract	**Extract Conc (%)**	**S. aureus**	**S. pyogenes**	**S. typhi**	**P. mirabilis**	**P. aeruginosa**	**E. coli**
Ethanol	100	7.39±0.01	12.25±0.21	9.73±0.04	7.55±0.07	10.52±0.02	9.50±0.00
	50	4.38±0.01	9.33±0.11	-	4.17±0.06	6.86±0.03	4.38±0.01
	25	-	7.20±0.28	-	-	5.45±0.07	-
Methanol	100	10.75±0.02	11.00±0.00	10.60±0.07	7.39±0.01	7.88±0.01	10.00±0.00
	50	6.30±0.14	9.75±0.01	5.30±0.04	-	2.62±0.01	5.18±0.06
	25	4.39±0.01	4.67±0.06	-	-	-	-
Aqueous	100	11.89±0.01	12.64±0.01	6.00±0.00	7.88±0.01	12.62±0.01	9.73±0.04
	50	7.55±0.07	7.40±0.03	5.45±0.07	-	11.22±0.12	8.14±0.01
	25	-	2.17±0.05	4.00±0.00	-	8.00±0.00	5.45±0.07
Control	100	14.3±0.14	14.00±0.00	10.23±0.04	9.39±0.01	13.07±0.09	11.44±0.08
	50	10.89±0.01	13.07±0.09	6.39±0.01	4.17±0.06	9.25±0.00	8.00±0.00
	25	8.91±0.04	9.62±0.02	5.30±0.04	-	8.40±0.00	6.00±0.00

Key: - = No inhibition, Value = Mean ± SD

3.4. Minimum Inhibitory Concentration of the Extracts on Test Bacteria

The minimum inhibitory concentrations (MICs) of the extracts against the bacteria are shown in Table 4. Among the test organisms, Staphylococcus aureus was very sensitive to the methanolic extract, with the least MIC of 25%. Streptococcus pyogenes was highly inhibited by both the ethanolic and methanolic extracts at 25% concentration. Pseudomonas aeruginosa recorded an MIC of 50% against the methanolic extract, while Salmonella typhi was the most resistant among the test bacteria, with an MIC of 100%. Proteus mirabilis was fairly sensitive to the ethanolic

and aqueous extracts at 50% concentration, while Escherichia coli was most susceptible to the aqueous extract with an MIC of 25%.

Table 4: Minimum Inhibitory Concentration (MIC) of the Extracts Against the Bacteria

		Minimum Inhibitory Concentration (MIC) (%)					
Extract	**Extract Conc (%)**	**S. aureus**	**S. pyogenes**	**P. aeruginosa**	**S. typhi**	**P. mirabilis**	**E. coli**
Ethanol	100	-	-	-	-	-	-
	50	-	-	+	+	-	+
	25	+	-	+	+	+	+
	MIC	50	25	100	100	50	100
Methanol	100	-	-	-	-	-	-
	50	-	-	-	+	+	-
	25	-	-	+	+	+	+
	MIC	25	25	50	100	100	50
Aqueous	100	-	-	-	-	-	-
	50	-	+	+	+	-	-
	25	+	+	+	+	+	-
	MIC	50	100	100	100	50	25

Key: + = Growth, - = No Growth,

3.5. Heavy Metal and Mineral Analysis of Crude Extract of B. pinnatum

The quantitative heavy metals and mineral contents of B. pinatum leaf are shown in Table 5. Potassium recorded the highest value among the minerals tested (7.899 ppm), followed by calcium (7.788 ppm) and Magnesium (6.893 ppm), while manganese had the least (0.021 ppm). Other minerals found in the leaves of the plant include zinc, sodium and magnesium, with recorded values of 0.9203, 3.574 and 0.021ppm respectively. Among the heavy metals, silver had the highest value (3.130 ppm), followed by nickel (1.038 ppm), while arsenic recorded the least (0.008ppm).

Table 5: The Quantitative Heavy Metal and Mineral Contents of B. Pinatum Leaf

Component	Concentration (ppm)
Lead	0.38
Cadmium	0.072
Zinc	0.9203
Copper	0.104
Chromium	0.265
Nickel	1.038
Mercury	0.191
Sodium	3.574
Silver	3.130
Manganese	0.021
Cobalt	0.028
Arsenic	0.008
Calcium	7.788
Aluminum	0.027
Selenium	0.677
Potassium	7.899
Molybdenum	0.028
Magnesium	6.893

4.0 Discussion

Photochemical analysis is valuable technique in assessing bioactive compounds in medicinal plants. The phytochemicals present in the Bryophyllum pinnatum leaf include alkaloids, glycoside, tannins, saponins, terpenoids, phenols, flavonoids, steroids, hydrogen cyanides, oxalates and phytates. Different quantities of these bioactive compounds have been reported by authors that worked on the plant material, depending on the solvents employed [18, 21, 22]. Some of these phytochemicals have been reported to possess some pharmacological potential. For instance, naturally occurring alkaloidsand their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties [23]. Alkaloids are capable of reducing headache associated with hypertension. Similarly, alkaloids use in the management of cold, fever and chronic catarrh has been reported. Alkaloids however could be toxic when consumed in bulk amount following their stimulatory effects, resulting in excitation linked with cellular and nervous disorders [24,25]. In this study, alkaloids were detected in moderate quantities across the solvents employed. This was however in agreement with previous studies.

Tannins are water soluble phenolic compounds capable of precipiting proteins from aqueous solution. This could explain its relative high presence in aqueous compared to the other extracts, as seen in this study. Tannins quicken the healing of wounds and inflamed mucous membranes [26]. They occur in all vascular plants. Tannins bind to proteins making them bio unavailable [27]. Quantitatively, the level of tannins recorded in this study was higher than that recorded by previous studies [18, 21, 28]. In the food industries, tannins are used to clarify wines, beer and fruit juices. It can also be used as coagulants in rubber production [29].

Similarly, B. Pinnatum leaf recorded relatively high saponins content in this study, especially in alcoholic extracts. This observation lends credence to the report by previous authors [30, 3, 32], but disagrees with other reports [21, 28]. These variations in trends of saponins contents of the plant material could be attributed to the different solvents used, methods of extraction, and species of the plant studied. Consumption of high levels of saponins has been associated with gastroenteritis [33]. In addition, saponins are known to produce an inhibitory effect on inflammation, precipitate and coagulate erythrocytes, hemolytic activity, cholesterol binding, bitter taste, and formation of foams in aqueous solutions [34, 35, 36].

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 [37]. The presence of flavonoids in appreciable amounts as observed in this study could be a confirmation of some of the pharmacological potentials of the plant. Cardiac glycosides are important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure [38]. The cardiac glycosides level reported in this study was low as similarly reported by [28], in their study on the extracts of Bryophyllum pinnatum grown in Anambra State, Nigeria.

The plant extracts have antibacterial potential against the tested bacteria, as could be observed from the zones of inhibition and minimal inhibitory concentration results. Generally, within each extract and the control, the susceptibility pattern of the test bacteria varied with the concentrations of the extracts. The aqueous extract was the most effective inhibitor of the test organisms among the extracts tested. The stronger extraction capacity of the aqueous extract as indicated in the phytochemical analyses results could have been responsible for the higher antimicrobial activity observed in this study.This observation contradicted the reported high effectiveness of ethanolic extract of Bryophyllum pinnatum leaf on the tested pathogenic bacteria compared to the other extracting solvents [39, 40].Furthermore, in the same study by Akinnibosun and Edionwe, Staphylococcus aureus and Pseudomonas aeruginosa showed the highest and least susceptibility

respectively in the same extract, as against Streptococcus pyogenes and Salmonella typhi respectively observed in this study. The differences in the test organisms and the variations in the concentrations of the extracts used could account for the differences in the observed effects.

In methanolic extract, Streptococcus pyogenes with the highest zone of inhibition across the concentrations was the most sensitive bacterium, while Proteus mirabilis was the least. In a study on antimicrobial activity of extracts of Bryophyllum pinnatum on selected pathogens, Klebsiella pneumoniae however was reported to be the most sensitive bacterium to the methanolic extract while Streptococcus pyogenes was the least [41]. Also in the same study, contrary to our observation in this study, methanol extract of B. pinnatum was reported to be most effective against the test organisms than the aqueous solvents. The positive control (Chloramphenicol) was more effective in inhibiting the growth of the tested bacteria at the tested concentrations than the extracts. However, while all the extracts performed well against the test organisms, the aqueous extract compared relatively well with the standard antibiotic that was used as a positive control. Similar result was observed while using Ciprofloxacin as a positive control [40].

Methanolic extract was he most effective against Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosawith the least MIC, while the aqueous extract was the most effective against Escherichia coli and Proteus mirabilis. In study on the antimicrobial activity of Bryophyllum pinnatum on selected pathogens, also reported that methaolic extract was the most effective against Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli.

The potassium content of the leaf was ≈ 7.90 ppm. This was much less than the value previously reported for the plant leaf (30.4 ± 17.17ppm) by [18].However as observed in this study, potassium has been reported as the most abundant mineral in agricultural products in Nigerian [42].Potassium is necessary in maintaining body weight; regulate water and electrolyte balance in the blood and tissues [43]. Calcium was the second most abundant macro element in this study. 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 concentration of sodium in the sample was low (3.574 ppm) and could explain the use of the plant in treating heart-related diseases by the natives. Excess sodium consumption results in hypertension [43].Zinc is a necessary trace element for protein and nucleic acid synthesis and normal body development [44]. It also stimulates the activity of vitamins, and the formation of red and white blood cells [45]. Zinc plays a role in improving male fertility. Zinc was detected in the plant leaf but at low concentration (0.9203 ppm). The magnesium content of the plants was

6.893ppm in this study. Magnesium plays fundamental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acids. It also plays a significant role in the intestinal absorption of electrolyte in the body. Its deficiency in man leads to severe diarrhea and persistent migraines [46]. Copper level in the plant samples under study is 0.104 ppm. 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 [47]. 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. Other minerals such as chromium were found to be present in appreciable quantities. This indicates that the plant is rich in minerals useful for proper physiological and metabolic functions of the body. Some of these micro- and macro- nutrients observed in this study have previously been reported in the plant [48].

Furthermore, heavy metals such as lead, zinc, copper, nickel, cadmium, chromium, silver, mercury, arsenic, cobalt, aluminum, molybdenum, selenium and nickel were found in the plant leaves. Though some of these heavy metals are known co-enzymes, others have no-known physiological functions in the body and therefore toxic when consumed in large amount [49]. In addition, the presence of some of these heavy metals in relatively high amounts in the plant leaf makes this plant a potential veritable tool in bioremediation of heavy metals contaminated soils. Similar assertion was made by previously [50].

5.0 Conclusion

The phytochemical composition of the ethanol, methanol and aqueous extracts of Bryophyllum pinnatum leaf of indicated the abundance of many secondary metabolites like flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides among othersin various amounts. The minerals and heavy metals contents of the plant leaf include calcium, potassium, magnesium, sodium, zinc, iron, lead, copper, nickel, cadmium, chromium, silver, mercury, arsenic, cobalt, aluminum, molybdenum, selenium and nickel. The clear zones of inhibition recorded in this study strongly indicate the plant's ability to inhibit bacterial growth, thus indicating it's as an antibacterial agent.

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