Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 13, Issue 2, 2025

Phytochemical Profile and Antimicrobial Activity of *Bryophyllum pinnatum* Leaf Extracts Against Otorrhea Isolates

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Accepted: *December 16, 2025.* **Published Online***: December 19, 2025*

ABSTRACT

Ear infections remain a global public health challenge, particularly in low- and middle-income countries where inadequate treatment can result in hearing loss, chronic morbidity, and increased healthcare costs. Rising antimicrobial resistance has intensified the search for alternative medicinal plants offering a promising avenue. *Bryophyllum pinnatum*, widely used in African traditional medicine, is reputed for its antimicrobial and anti-inflammatory properties. This study investigated the phytochemical constituents and antimicrobial potential of *B. pinnatum* extracts against bacterial isolates obtained from ear pus. Its leaves were extracted using hexane, butanol, ethylacetate and water. Results of phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, steroids, and carbohydrates, while anthraquinones were absent. Antimicrobial activity was assessed against *Staphylococcus spp.*, *Proteus spp.*, and *Klebsiella spp*. using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Crude and hexane extracts demonstrated the strongest antimicrobial activity, with inhibition zones ranging from 10–19 mm and MIC values between 25–50 mg/mL. Aqueous, butanol, and ethyl acetate extracts exhibited weak or no activity. These findings provide scientific validation for the traditional use of *B. pinnatum* in managing infections and highlighted its potential as a source of novel antimicrobial agents.

Key words: *Bryophyllum pinnatum.* phytochemical screening, anti-microbial resistance, ear infection, traditional medicine, Minimum inhibition concentration, Minimum bactericidal concentrations.

INTRODUCTION

Ear infections, particularly *Otitis media* and *Otitis externa,* are prevalent health conditions that affect millions of children worldwide. These infections are often caused by pathogenic microorganisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which lead to the accumulation of pus in the ear [1,2]. This condition, if left untreated, may result in complications like hearing loss, mastoiditis, or intracranial infections [3]. Despite the availability of antibiotics, the rising prevalence of multidrug-resistant bacteria poses a significant challenge to effective treatment [4].

Traditional medicine, which relies on plant-based remedies, has been an integral part of healthcare systems in many cultures [5,6]. One such plant is the *Bryophyllum pinnatum* a succulent belonging to the family *Crassulaceae*, commonly referred to as the "miracle leaf" or "life plant." The Hausa speaking people amongst which this research was conducted called it ‘’*Sutura”*. The plant has been used in folklore medicine to treat various ailments, including wounds, ulcers, infections, and inflammation [7, 8].

Studies indicate that the bioactive compounds in *Bryophyllum pinnatum*, such as alkaloids, flavonoids, tannins, and saponins, possess significant antimicrobial and anti-inflammatory properties [9,10]. However, specific research on the use of the plant to tests its activity against disease causing pathogens are very scanty as such this study aims to investigate the phytochemical profile of *Bryophyllum pinnatum* (Plate 1) and assess its antimicrobial efficacy against pus isolates obtained from infected ears. By combining traditional knowledge with scientific methods, this research could contribute to the development of novel therapeutic agents for combating ear infections.

Plate 1: *Bryophyllum pinnatum*

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh leaves of *Bryophyllum pinnatum* were collected from a garden in Kaduna Metropolis , Nigeria, in November 2024. The plant was authenticated by a taxonomist at the Department of Biological Science, Kaduna State University Herbarium. The voucher number for the specimen is KASU/BSH/239.The leaves were washed, air-dried at room temperature (7–10 days), and ground into fine powder before extraction.

Extraction

The powdered leaves were placed in a Soxhlet apparatus and extracted with four solvents of increasing polarity: butanol, ethylactate, hexane, and distilled water. Each solvent was used for a period of 6 hours to ensure efficient extraction of the bioactive compounds. The resulting extracts were stored at 4 °C in airtight containers until they were used for further analysis [11].

Phytochemical Screening

Qualitative screening of extracts for alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, terpenoids, carbohydrates, and anthraquinones was performed using standard protocols [12 - 14].

Test for carbohydrates

Molisch's test

To a small portion of the extract in a test tube, 3 drops of Molisch's reagent were added followed by concentrated sulfuric acid by the side of the test tube. The formation of a reddish colored ring at the interface indicated the presence of carbohydrates.

Test for saponins

Frothing test

About 10 ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The solution was allowed to stand for 5 minutes. The formation of a persistent froth indicated the presence of saponins.

Test for flavonoids

Ammonium test

Oil (0.5 g) was heated with 10 ml of ethylacetate in boiling water for 3 min. The mixture was filtered differently and the filtrates were used for the following tests: One portion of the filtrate was shaken with 1 ml of dilute ammonia solution (1%). A yellow coloration at the ammonia layer indicates the presence of flavonoids. The second portion was shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow coloration. A yellow precipitate indicated the presence of flavonoids

Test for phenolic compound

Ferric chloride

The extract (0.5 g) was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group.

Test for steroids and terpenoids

Concentrated Sulphuric Acid Test

About 9 ml of ethanol was added to 1 ml of the extract and then refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5 ml was added to the concentrated solution, the mixture was allowed to stand for 1 h and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of the chloroform extract in a test tube, 1 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown interface showed the presence of steroids. To another 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 min on a water bath. A grey colour indicated the presence of terpenoids.

In addition, Salkowski test for the presence of terpenoids was also carried out as follows:

Salkowski test

A small portion of the extract was dissolved in 2ml of chloroform, 3 drops of concentrated sulphuric acid were added at the side of the test tube. A red brown coloration at the interface indicated the presence of terpenoids.

Test for tannins

The oil (0.5 g) was boiled with 5 ml of 45% ethanol for 5 min. The mixture was cooled and filtered. The filtrate was subjected to the following tests:

Ferric chloride test

The filtrate (1 ml) was diluted with distilled water and added 2 drops of ferric chloride. A transient greenish to black color indicated the presence of tannins

Lead sub-acetate test

To a small portion of the extract, 4 drops of lead sub-acetate solution was added, the formation of a cream colored precipitate indicated the presence of tannins.

Test for alkaloids

The extract sample (0.5 g) was boiled with 5 ml of 2% HCl on a steam bath. The mixture was filtered and 1 ml portion of the filtrate was measured into three test tubes. Each of the 1 ml filtrate was treated with 2 drops of the following reagents:

Dragendorff's reagent. Appearance of red precipitate indicated the presence of alkaloids.

Mayer's reagent. Appearance of creamy-white colored precipitate indicated the presence of alkaloids.

Wagner's reagent. Appearance of a reddish-brown precipitate indicated the presence of alkaloids.

Test for Reducing Sugar

Portion of the extract (0.5 ml) was shaken vigorously with 5 ml of distilled water and filtered. To the filtrate was added equal volumes of Fehling solutions A and B and shaken vigorously. A brick red precipitate indicated the presence of reducing sugars.

Test of Anthraquinones

Bontrager's test

A small portion of the extract was dissolved in 5ml chloroform, shaken and filtered. To the filtrate, an equal volume of 10% ammonium solution was added with continuous shaking. A bright pink colour in the aqueous upper layer indicated the presence of anthraquinone.

Test for Cardiac Glycosides

Sulphuric acid test

Dilute sulphuric acid (5 ml) was added to 0.5 ml of the test extract in a test tube and boiled for 15 min in a water bath. It was then cooled and neutralized with 20% potassium hydroxide solution. A mixture, 10 ml of equal parts of Fehling's solution A and B was added and boiled for 5 min. A denser red precipitate indicated the presence of glycoside.

Keller-Kiliani test

A small portion of the extract was dissolved in 1ml glacial acetic acid containing traces of ferric chloride solution. The solution was then transferred into a dry test tube to which an equal volume of sulphuric acid was added; a brown ring obtained at the interface indicated the presence of deoxy-sugars (digitoxose).

Microbial Isolation and Identification

Ear pus samples were collected aseptically from patients diagnosed of ear problem at a hospital in Tudun Wada, Kaduna South Local Government Area of Kaduna State, Nigeria. The samples were inoculated onto selective media (nutrient agar, MacConkey agar, mannitol salt agar) and incubated at 37 °C for 24 h. Colonies were identified by Gram staining and standard biochemical tests as indicated in Table 1 [15].

Antimicrobial assay

The agar well diffusion method was employed to assess antimicrobial activity against *Staphylococcus spp., Proteus spp., and Klebsiella spp*. Extract concentrations of 12.5–100 mg/mL were tested. Ciprofloxacin served as the positive control. Minimum inhibitory concentration and minimum bactericidal concentration were determined following Eucast guidelines [16].

RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, terpenoids, and carbohydrates, whereas anthraquinones were absent as indicated in Table 1.

Table 1: Phytochemical Composition of *Bryophyllum pinnatum*

Phytochemical	Test	Observation	Inference
Alkaloids	Dragendorff's reagent	Formation of creamy whit ppt indicated presence of alkaloids	+
	Wagner's test	Formation of whitish ppt indicated the presence of alkaloid	+
Flavonoids	Alkaline reagent test	Yellow color was formed which became colorless indicating the presence of flavonoids	+
Tannins	Ferric Chloride test	Greenish-black or blue color indicated the presence tannins	+
	Lead sub-acetate	Formation of creamy coloured ppt indicated the presence of tannins	+
Saponins	Frothing test	Formation of foam indicated the presence of saponins	+
Phenols	Lead acetate test	Yellow colored ppt indicated the presence of phenol	+
	Keller-killiani test	Formation of a reddish-brown ring between the layers indicated the presence of Phenols	+
Glycosides	Sulphuric acid test	Formation of dense red ppt indicated the presence of glycosides	+
Anthraquinones	Bontrager's test	No color change	-
Terpenoids	Liebermann burchard test	Green color was formed	+

		Indicating the presence of terpenoids	+
Carbohydrates	Molisch's test	Formation of reddish colored interfacial ring	+
Steroids	Salkowski's test	Reddish-brown color formed indicating the presence of steroids	+

Key: (+) = present, (-) = absent, (ppt) = precipitate

Antimicrobial Activity

The antimicrobial assay of the crude, ethylacetate, aqueous, hexane and butane extracts exhibited notable antimicrobial activity, with inhibition zones ranging from 10–19 mm against *Staphylococcus spp*., *Proteus spp.*, and *Klebsiella spp*. as indicated in table 3. MIC values ranged between 25–50 mg/mL, while MBC values were observed at 50 mg/mL for *Klebsiella spp*. Aqueous, butanol, and ethyl acetate extracts showed little or no activity as shown in Table 2.

Table 2: Gram's Reaction, Cell morphology and Bacterial Characterization of Bacterial Isolates

Sample code	Gram's reaction	Cell morphology	Indole	MR	VP	Citrate	TSI	Urease	Motility	Oxidase	Catalase	Presumptive organism
S1	+	Cocci	-	-	+	-	-	+	-	-	+	*Stapylococcus spp*
S2	+	Cocci	-	-	+	-	-	+	-	-	+	*Staphylococcus spp*
S3	-	Rods	+	+	-	+	k/A	+	-	-	-	*Proteus spp*
S4	-	Rods	+	+	-	-	K/A	+	-	-	-	*Proteus spp*
S5	-	Rods	-	+	+	+	A/A	+	-	-	-	*Klebsiella spp*
S6	+	Cocci	-	-	+	-	K/K	+	-	-	+	*Stapylococcus spp*
S7	+	Cocci	-	-	+	-	K/K	+	-	-	+	*Staphylococcus spp*
S8	-	Rods	-	-	+	+	A/A	+	-	-	-	*Klebsiella spp*
S9	-	Rods	+	+	-	-	K/A	-	-	-	-	*Proteus spp*

Key: (+) present, (-) Absent, (K/A) alkaline/acid reaction (A/A)= acid/acid reaction, (K/K) alkaline/alkaline reaction, (TSI)= glucose, sucrose and lactose, (MR) Methyl red, (VP)=voges-proskauer, (SPP)= species, (S) = Sample

Determination of inhibitory activity (sensitivity test) of crude, aqueous, hexane, butane, ethyl acetate extracts on the test organisms

Table 3 shows the crude extract exhibited the largest zones of inhibition across all bacterial strains, followed by the hexane extract. Negative zone of inhibition for aqueous, ethyl acetate and butane extract indicates resistance by the organisms. The result showed that at 100 mg/ml concentration, there is high level of antimicrobial activity compared to other concentrations. The result further suggests that hexane extract of *Bryophyllum pinnatum* possesses the strongest antimicrobial properties due likely because of its concentration of bioactive compounds such as tannins, phenols, saponins known for their antimicrobial properties.

Table 3: Antimicrobial Activity of Crude, Ethylactate, Aqueous, Butane extracts of *Broyphyllum pinnatum*

sample no	test organism	conc. (mg/ml)	zone of Inhibition (mm)				cont(mm)
			crude	ethylacetate	aqueous	butane	hexane	
S1	*Staphylococcus spp*	100	15	-	-	-	11	25
		50	15	-	-	-	-	
		25	11	-	-	-	-	
		12.5	-	-	-	-	-	
S2	*Staphylococcus spp*	100	12	-	-	-	-	26
		50	15	-	-	-	--	
		25	-	-	-	-	-	
		12.5	-	-	-	-	-	
S3	*Proteus spp*	100	12	-	-	-	10	27
		50	10	-	-	-	-	
		25	-	-	-	-	-	
		12.5	-	-	-	-	-	
S4	*Proteus spp*	100	13	-	-	-	-	27
		50	10	-	-	-	-	
		25	-	-	-	-	-	
		12.5	-	-	-	-	-	

S5	*Klebsiella spp*	100	19	-	-	-	17	32
		50	16	-	-	-	16	
		25	12	-	-	-	12	
		12.5	-	-	-	-	-	
S6	*Staphylococcus spp*	100	13	-	-	-	-	25
		50	10	-	-	-	-	
		25	-	-	-	-	-	
		12.5	-	-	-	-	-	
S7	*Staphylococcus spp*	100	18	-	-	-	-	27
		50	14	-	-	-	-	
		25	12	-	-	-	-	
		12.5	-	-	-	-	-	
S8	*Klebsiella spp*	100	16	-	-	-	-	26
		50	13	-	-	-	-	
		25	10	-	-	-	-	
		12.5	-	-	-	-	-	
S9	*Proteus spp*	100	13	-	-	-	-	29
		50	10	-	-	-	-	
		25	-	-	-	-	-	
		12.5	-	-	-	-	-	

Key: (mg/ml)

= milligram/milliliter, (mm)= millimeter spp= species,

Ear pathogens: *Staphylococcus*, *Klebsiella spp*, and *proteus spp*. The results of the antimicrobial testing using the agar well diffusion method and the minimum inhibitory concentration method are presented in Table 3. The size of the zone of inhibition is directly proportional to the level of antimicrobial activity, a larger zone of inhibition shows more antimicrobial activity.

Determination of minimum inhibitory concentration and minimum bacteriocidal concentration of the extract against the test organisms

The MIC values for the extracts of *Bryophyllum pinnatum* were determined using agar well diffusion method. The results of the MIC and MBC values for various bacterial pathogens are presented in Table 4.

Table 4: Concentration of the Crude Extract (mg/ml)

Sample code	Test Organisms	MIC (mg/ml)	MBC (mg/ml)
CR1	*Staphylococcus spp*	50	NIL
CR2	*Staphylococcus spp*	50	NIL
CR3	*Proteus spp*	50	NIL
CR4	*Proteus spp*	50	NIL
CR5	*Klebsiella spp*	25	50
CR6	*Staphylococcus spp*	50	NIL
CR7	*Staphylococcus spp*	25	50
CR8	*Klebsiella spp*	25	50
CR9	*Proteus spp*	50	NIL
HX1	*Staphylococcus spp*	50	NIL
HX2	*Klebsiella spp*	25	50

Key: (SPP) =Species, (CR)= Crude, HX= Hexane

The results from the MIC and MBC determination further support the conclusion that *Bryophyllum pinnatum* extracts, particularly the in the crude and hexane extract, possess significant antimicrobial potential. Although the MIC values of the plant extracts were lower than those of the standard antibiotics (Ciprofloxacin), which reflects the potency of the plant extracts but also suggests that further optimization of extraction methods might be necessary for more efficient antimicrobial activity. The result from the MIC and MBC therefore showed that both the crude and hexane extracts are bacteriostatic and bactericidal.

The findings from this study provide strong evidence supporting the antimicrobial potential of *Bryophyllum pinnatum* against common ear pathogens. The plant extracts demonstrated inhibitory effects on both Gram-positive and Gram-negative bacteria, which are responsible for a variety of ear infections. The phytochemical profile of *Bryophyllum pinnatum* is consistent with its traditional medicinal uses. Alkaloids, flavonoids, saponins, tannins, and phenols are known to possess antimicrobial and anti-inflammatory properties. The presence of glycosides in the methanol extract is also noteworthy, as these compounds are commonly associated with antimicrobial activity [17].

The results of the agar well diffusion and MIC tests indicated that crude and hexane extracts of *Bryophyllum pinnatum* exhibited the strongest antimicrobial activity compared to butanol, ethyl acetate and aqueous extracts. This can be attributed to the higher concentration of bioactive compounds extracted by which is known for its ability to extract non-polar compounds effectively and some polar compounds to some extent [10, 18,19].

The significant activity against both Gram-positive (*Staphylococcus spp*) and Gram-negative (*klebsiella spp*) bacteria suggests that *Bryophyllum pinnatum* may be a broad-spectrum antimicrobial agent while the plant extracts showed promising activity but they were less effective than the standard antibiotics in terms of MIC. This is common for plant-based antimicrobial agents, which are often less potent than synthetic drugs due to the complexity of their bioactive compounds [1, 20].

However, the antimicrobial activity observed in this study suggests that *Bryophyllum pinnatum* could serve as a valuable adjunct to traditional treatments, particularly in cases of antibiotic-resistant infections. The antimicrobial properties of *Bryophyllum pinnatum* highlight its potential for use in managing ear infections caused by common pathogens, including antibiotic-resistant strains [21 - 23]. Given the growing concern about antibiotic resistance, this plant could provide an alternative or complementary treatment option in the management of ear infections, particularly in regions where access to antibiotics is limited. The outcomes also align with reports of *B. pinnatum's* traditional use for wound healing and infection management [7,24]. Further bioassay-guided fractionation and *in-vivo* studies are necessary to isolate active compounds and validate therapeutic applications.

CONCLUSION

This study provides scientific evidence for the antimicrobial potential of *Bryophyllum pinnatum*, against ear pus pathogens. The presence of diverse phytochemicals likely underpins its bioactivity although less potent than conventional antibiotics. *B. pinnatum* represents a promising candidate for developing alternative therapies, especially in regions burdened by antimicrobial resistance.

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