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ANTIBACTERIAL, ANTIOXIDANT, AND PHYTOCHEMICAL PROPERTIES OF BRYOPHYLLUM PINNATUM (LAM.) LEAF EXTRACTS AGAINST PATHOGENIC BACTERIA

MANFAAT ANTIBAKTERI, ANTIOKSIDAN DAN FITOKIMIA EKSTRAK DAUN BRYOPHYLLUM PINNATUM (LAM.) TERHADAP BAKTERI PATOGEN

Elizabeth Tomilayo Akinterinwa [*1,2], Babayemi Olawale Oladejo [2], Muftau Kolawole Oladunmoye [2]

[1] *Microbiology Program, College of Natural and Applied Sciences, Wesley University Ondo, Ondo State, Nigeria*
[2] *Department of Microbiology, School of Life Sciences, Federal University of Technology Akure, Ondo State, Nigeria*

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A B S T R A C T

Background: *Bryophyllum pinnatum (Lam.) (Family: Crassulaceae), also known as Kalanchoe pinnatum, is a perennial herb, 3 to 5 meters tall, with opposed glabrous leaves. It is used to treat various illnesses due to its many health benefits.* ***Purpose:*** *This study aims to investigate the biological importance of B. pinnatum leaf extracts against some pathogenic bacteria.* ***Method:*** *Freshly harvested leaves of B. pinnatum were collected, air-dried, pulverized to powder and stored in air-tight containers using standard methods. Extracts were obtained from the powder using ethanol, ethyl acetate and distilled water. Pathogenic bacterial isolates were collected and their identity were confirmed using colonial and biochemical tests. The extracts were screened for antibacterial, antioxidant and phytochemical potentials using standard methods.* ***Result:*** *The highest percentage yield was obtained with the ethanol extract (12.2%) of B. pinnatum and the lowest yield (5.3 %) was obtained with the ethyl acetate extract. The ethanol extract was effective against eight (8) out of the thirteen test isolates, while the aqueous extract had the lowest activity and was effective against only two bacteria (Pseudomonas aeruginosa and Haemophillus influenzae). The lowest MIC observed with the three extracts is 25 mg/ml. B. pinnatum leaf extracts possess high antioxidant properties that are concentration dependent. The phytochemical constituents that were recorded in this study include saponins, terpenoids, steroids, tannins and glycosides. Steroid was present in only the ethanol extract but absent in the other two.* ***Conclusion:*** *B. pinnatum leaves have antibacterial, antioxidant and phytochemical properties and could be further utilized to manage bacterial infections.*

A B S T R A K

Latar belakang: *Bryophyllum pinnatum* (Lam.) (Famili: Crassulaceae) yang juga dikenal sebagai *Kalanchoe pinnatum* (cocor bebek) adalah tanaman herbal setinggi 3 - 5 meter yang memiliki daun gundul yang melintang, dengan banyak manfaat kesehatan termasuk untuk mengobati berbagai penyakit. **Tujuan:** Penelitian ini bertujuan untuk mengetahui pentingnya ekstrak daun *B. pinnatum* terhadap beberapa bakteri patogen. **Metode:** Daun *B. pinnatum* yang baru dipanen dikumpulkan, dikeringkan dengan udara, dihaluskan menjadi bubuk, dan disimpan dalam wadah kedap udara menggunakan metode standar, ekstrak diperoleh dari bubuk menggunakan etanol, etil asetat, dan air suling. Isolat bakteri patogen dikumpulkan dan identitasnya dikonfirmasi menggunakan uji kolonial dan biokimia. Ekstrak disaring untuk potensi antibakteri, antioksidan, dan fitokimia menggunakan metode standar. **Hasil:** Persentase hasil tertinggi diperoleh dengan ekstrak etanol (12,2%) dari *B. pinnatum* dan hasil terendah (5,3%) diperoleh dengan ekstrak etil asetat. Ekstrak etanol efektif terhadap delapan (8) dari tiga belas isolat uji, sedangkan ekstrak air memiliki aktivitas terendah dan hanya efektif terhadap dua bakteri (*Pseudomonas aeruginosa* dan *Haemophillus influenzae*). MIC terendah yang diamati dengan tiga ekstrak adalah 25 mg/ml. Ekstrak daun *B. pinnatum* memiliki sifat antioksidan tinggi yang bergantung pada konsentrasi. Konstituen fitokimia yang tercatat dalam penelitian ini meliputi saponin, terpenoid, steroid, tanin, dan glikosida. Steroid hanya terdapat dalam ekstrak etanol tetapi tidak terdapat dalam dua ekstrak lainnya. **Kesimpulan:** Daun *B. pinnatum* memiliki sifat antibakteri, antioksidan, dan fitokimia dan dapat digunakan lebih lanjut untuk mengelola infeksi bakteri.

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Correspondence:
Elizabeth Tomilayo Akinterinwa

E-mail :
idowutomilayo@yahoo.com

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INTRODUCTION

A rapidly-growing global health challenge is the loss of potency of conventional drugs that were once known to treat certain diseases effectively. This loss of potency is very common with antibacterial drugs or antibiotics, mainly because of the resistance of many bacteria to these drugs. The World Health Organization (WHO) has described it as the most urgent problem medical science faces. The existence of resistant microorganisms to antibiotics, which has also led to the presence of resistant genes in the environment is mainly attributed to the misuse of antibiotics and the various methods for adaptation utilized by these microorganisms (Huang *et al*., 2016). In developing countries, other problems associated with the use of conventional drugs in addition to antibiotic resistance are high cost, inaccessibility, and the side effects associated with the use of these drugs. These have made it necessary to search for new organic compounds that have antibacterial and antioxidant properties in order to potentially find raw materials for the semi-synthesis of new drugs.

Fortunately, the discovery and utilization of medicinal plants brought significant relief to the world for managing and controlling various diseases. A medicinal plant is simply defined as a plant in which one or more of its parts contain certain compounds that have the potential to be used in the manufacture of drugs or in new drug design (Mani, 2016). These compounds are referred to as secondary metabolites or phytochemicals, and they include terpenes, alkaloids, flavonoids, bioflavonoids, benzophenones, xanthones, tannins, saponins, cyanates, oxalate, anthraquinones, and phenylpropanoids (Daniel *et al*., 2020; Sanchita and Sharma, 2018). Some medicinal plants contain phenolic compounds, which possess antioxidant properties and may be essential to protecting cells against oxidative damage caused by free radicals (Daniel *et al*., 2020). They also serve as ion chelators and reduce agents.

According to a statement from the WHO, 80% of the developing world depends on medicinal plants (Khan *et al*., 2019). The numerous advantages that medicinal plants have over conventional drugs have contributed immensely to the continuous search for new ones as well as further exploration of other unknown potentials of those that have already been used. These advantages mainly include structural diversity (Harvey *et al*., 2015) and unique mechanisms of action (Lewis, 2020), among others. Several reports have shown that extracts from medicinal plants possess antimicrobial, anti-inflammatory, and antioxidant properties. According to Porras *et al*. (2021), approximately 183 plant-based natural products, which serve as a potential source of antibacterial lead compounds and can be used as substitutes for synthetic drugs in the recent antibiotic resistance era, have been analyzed. A very good example of such a medicinal plant is *Bryophyllum pinnatum* (Lam.).

Bryophyllum pinnatum (Crussulaceae) is a succulent glabrous herb that is 0.3 – 1.2 m tall (Azuonwu, 2018). It is native to Madagascar and Southern Africa and grows mainly in the tropics (Nagaratna and Hegde, 2015). *B. pinnatum* is commonly called an air plant, a life plant, a love plant, or a miracle leaf. In Nigeria, it is known as 'odaa opue' by the Igbo people and 'ewe abamoda' or 'odundun' by the Yorubas (Oladejo *et al*., 2021). *B. pinnatum* leaves are used to treat respiratory tract infections and many other bacterial infections. As a plant that is easy to cultivate and has also been utilized traditionally for the treatment of various illnesses, *B. pinnatum* leaves extracts could possess various bioactive compounds that can serve as good alternative to antibiotics in order to combat the global challenge of antibiotic resistance. This research investigates the bioactive compounds in extracts of *B. pinnatum* leaves which contributes to their antibacterial and antioxidant potentials.

MATERIAL AND METHOD

Collection of bacterial isolates

The bacterial isolates used in this study were obtained from the culture collection unit of the Department of Microbiology Research Laboratory, Federal University of Technology, Akure (FUTA). The isolates included *Staphylococcus aureus* (2), *Streptococcus pneumoniae* (2), *Streptococcus pyogenes* (2), *Pseudomonas aeruginosa* (2), *Haemophillus influenzae* (2), *Klebsiella pneumoniae* (2) and *Escherichia coli* (1). Colonial, morphological and biochemical tests were used for the identification of bacterial isolates.

Collection of plant samples

Fresh *B. pinnatum* leaves were obtained from adult plant specimens in residences in Ado-Ekiti, Ekiti State, Ilara-Mokin, Ondo State and Ilesa, Osun state, Nigeria. The plant materials were identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria with voucher number FUTA 0377. The plants were washed with clean running tap water, air-dried at 25 ^{0}C, pulverized into powder using an electric blender and stored in air-tight containers.

Preparation of *Bryophyllum pinnatum* leaves extracts

Extracts from *B. pinnatum* leaves were obtained according to the methods of (Adeleke *et al*., 2007) with ethanol, distilled water and ethyl acetate. One hundred grams of the powdered leaves were steeped in 1 L of each of the three solvents for 72 hours with regular agitation. The extracts obtained were sterilized using a syringe filter with a pore size of 0.22 µm and preserved in sterile air-tight containers at 4 ^{0}C for further use. The recovery rate of extracts was calculated using Formula 1.

$$\text{Percentage recovery/yield of extract} = \frac{\text{Weight of extract recovered after extraction}}{\text{Initial weight of plant before extraction}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots (1)$$

Standardization of inoculum and antibacterial assay of the extracts

The bacterial isolates were standardized according to the method described by Adeleke *et al*. (2007). The agar well diffusion method was used to test the effectiveness of the plant extracts against the bacterial isolates according to Kirmani *et al*. (2024). The standardized bacterial isolates were aseptically inoculated on the surface of sterile Mueller Hinton Agar (MHA) plates with the aid of a sterile swab stick by spread method. A sterile cork borer was used to bore 8 mm diameter wells in the culture medium to each of the wells, 0.5 ml of each of the extracts at 200 mg/ml concentration were added. The plates were left to stand on the laboratory bench for 30 minutes and then incubated for 24 hours at 37 ^{0}C. Clear inhibition zones were measured and recorded.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests

The extracts' minimum inhibitory concentration and minimum bactericidal concentration of the extracts were determined using the method adopted by Adeleke *et al*. (2007). The double-fold dilution method was used to prepare different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml) of the plant extracts from the initial 200 mg/ml and tested them against the bacterial isolates. The least concentration of the extracts that do not allow any growth of the test isolates gives the MIC. The tubes from the MIC test were plated to check for which of the concentrations that have completely prevented further growth of the test isolates, which was recorded as the MBC.

Antioxidant activity of the plant extracts

Ferric reducing property of the extracts

The reducing property of the extracts was determined by according to the method described by Daniel *et al*. (2020) with slight modification. Equal amounts (0.25 ml) of the extract, sodium phosphate buffer (200 mM at pH 6.6), and coulometric Karl-Fisher (KFC: 1%), were mixed together and incubated at 50 °C for 20 minutes. Trichloroacetic Acid (TCA: 10%) was further added to the mixture, before the mixture was centrifuged (2000 rpm) for 10 minutes. The supernatant (1 ml) was mixed with 1 ml of $FeCl_3$ solution (0.1%), and the absorbance was measured at 700 nm using a UV-visible spectrophotometer.

Free radical scavenging ability of the extracts

This was carried out using 1, 1-diphenyl-2-picryhydrazyl (DPPH), according to the method described by Kirmani *et al*. (2024). One milliliter of the extract was mixed with 1 ml of the 0.4 mM methanolic solution of the DPPH and the mixture was left in the dark for 30 minutes. The absorbance was measured at 516 nm.

Determination of Fe^{2+} chelation activity

The ability of the extract to chelate Fe^{2+} was determined using the method described by Oyeniran *et al.* (2021) with slight modifications as follows: $FeSO_4$ solution (150 mM) in a mixture of Tris-HCl (168 ml of 0.1 M, pH 7.4) and saline solution (218 ml), and the extract, was prepared, and the mixture was made up to 1 L with distilled water. The mixture was incubated at 37 °C for 5 minutes, and 13 ml of 1, 10 phenantroline was added. The absorbance of the resultant mixture was read at 510 nm.

Quantitative phytochemical screening of *B. pinnatum* leaf extracts

Determination of tannins

Each extract was finely ground and 0.2 g of each was poured into 50 ml sample bottles. Ten milliliters of 70% aqueous acetone were added and properly covered. The bottles were put in an ice bath shaker and shaken for 2 hours at 30 ^{0}C. Each solution was then centrifuged and the supernatant was stored in ice. A fixed volume of 0.2 ml of each solution was pipetted into the test tube, followed by the addition of 0.8 ml of distilled water. Standard tannin acid solutions were prepared by diluting 0.5 mg/ml stock solution, which was then made up to 1 ml with distilled water. Half a mililitre of Folin ciocalteau reagent was added to the samples and standards, followed by the addition of 2.5 ml of 20% Na_2CO_3. The solution was vortexed and

allowed to incubate for 40 minutes at room temperature. Absorbance was measured at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve (Ajuru *et al*., 2018).

Determination of saponins

The spectrophotometric method was used for saponin determination. Two grams of extracts were weighed into a beaker and 100 ml of Isobutyl alcohol was added. The mixture was shaken for 5 hours to obtain a homogeneous mixture. The mixtures were filtered, and 20 ml of saturated magnesium carbonate solution (40%) was added to the filtrate and shaken to homogenize. The suspension obtained was filtered to obtain a colorless filtration. Aliquot (1 ml) of the filtrate was added to 2 ml of 5% $FeCl_3$ solution, and the mixture was made up to mark in 50 ml volumetric flask with distilled water. This mixture allowed to equilibrate for 30 minutes and develop colour. Thereafter, the absorbance was measured at 380 nm against blank (Ajuru *et al*., 2018).

Determination of alkaloids

The extracts were weighed (5 g) into beakers and 200 ml of 10% acetic acid in ethanol was added and allowed to equilibrate for 4 minutes. These extracts were filtered and concentrated (to about one-quarter of the original volume) in a water bath. Concentrated ammonium hydroxide was added dropwise to the samples until complete precipitation was achieved. The precipites were collected after dicountation, washed with dilute ammonium hydroxide and dried to obtain alkaloids (Daniel *et al*., 2020).

Determination of cardiac glycosides

The procedure described by Adeleke *et al*. (2007) was used. The extracts (10 mL) were transfered into a 250 ml conical flask. Chloroform (50 ml) was added, shaken for 1 hour, and filtered. Pyridine (10 ml) and 2 ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10 minutes. NaOH (3 ml of 20% solution) was then added to develop a brownish yellow color. The glycosides standard (Digitoxin) concentration (0 – 50 mg/ml) was prepared from the stock solution and the absorbance of the solutions obtained was extrapolated at 510 nm.

Determination of terpenoids

The procedure described by Oseni *et al*. (2021) with slight modifications was used. The extracts (0.5 g) were weighed into conical flasks. Chloroform and methanol (2:1) were added and the mixture was shaken thoroughly and allowed to equilibrate for 15 minutes at room temperature (25 °C). The suspension was centrifuged at 3000 rpm, the supernatant was discarded and the precipitate was re-washed with the chloroform-methanol (2 : 1) and then centrifuged again. The precipitate was dissolved in 40 ml of 10% SDS solution. Ferric chloride (0.01M: 1 ml) was then added and equilibrated for 30 minutes before the absorbance was taken at 510 nm. The standard terpenoid (alpha terpineol) concentration ranging from 0 - 5 mg/ml from the stock solution was used in the extrapolation of the concentration curve.

Determination of steroids

Steroid was determined by weighing the extracts (5 g) into conical flasks, where 50 ml of pyridine was added to it and shaken for 30 minutes at room temperature (25 °C). Copper (I) oxide (250 mg/ml) was added and the incubate was allowed to continue for 1 hour in the dark. The absorbance was measured at 350 nm against reagent blank Adeleke *et al*. (2007).

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20. The one-way ANOVA test was used to determine the statistical significance in the zones of inhibition of the extracts. p-value < 0.05 was considered significant.

RESULT

The extraction of bioactive compounds, or phytochemicals, from medicinal plants is crucial for effective drug development. Table 1 shown percentage recovery of *B. pinnatum* leaves extracts. Table 2 shown zones of inhibition produced by the plant extracts against the test bacterial isolates. Table 3 shown MIC of the plant extracts against the bacterial isolates. Table 4 shown MBC of the plant extracts against the test bacterial isolates. Table 5 shown phytochemical (quantitative) constituents of *B. pinnatum* leaves extracts. Figure 1 shown Fe^{2+} chelating (%) property of the plant extracts, Figure 2 shown free radical-scavenging (%) property of the extracts and Figure 3 shown Ferric Reducing/Antioxidant Power (FRAP) (mg/g) of the plant extracts.

Table 1. Percentage recovery of *Bryophyllum pinnatum* leaf extracts

Solvent	Original weight/input (g)	Extracted weight/output (g)	Percentage yield (%)	Colour of extract
BE	100	12.2	12.2	Greenish black
BEA	100	5.3	5.3	Yellowish black
BW	100	8.1	8.1	Brownish black

BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves

Table 2. Zones of inhibition produced by the plant extracts against the test bacterial isolates

Isolate	BW (mm)	BE (mm)	BEA (mm)
Staphylococcus aureus	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
Staphylococcus aureus	0.00 ± 0.00^{c}	24.00 ± 1.00^{a}	0.00 ± 0.00^{d}
Streptococcus pyogenes	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
Streptococcus pyogenes	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	22.50 ± 0.50^{a}
Streptococcus pneumoniae	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
Streptococcus pneumoniae	0.00 ± 0.00^{c}	22.50 ± 0.50^{a}	0.00 ± 0.00^{d}
Pseudomonas aeruginosa	0.00 ± 0.00^{c}	18.00 ± 1.00^{bc}	20.50 ± 0.50^{b}
Pseudomonas aeruginosa	16.50 ± 0.50^{b}	18.50 ± 0.50^{bc}	13.50 ± 0.50^{c}
Haemophilus influenzae	0.00 ± 0.00^{c}	14.00 ± 1.00^{d}	0.00 ± 0.00^{d}
Haemophilus influenzae	21.00 ± 1.00^{a}	19.00 ± 1.00^{b}	12.50 ± 0.50^{c}
Klebsiella pneumoniae	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	22.00 ± 1.00^{a}
Klebsiella pneumoniae	0.00 ± 0.00^{c}	19.50 ± 0.50^{b}	23.00 ± 1.00^{a}
Escherichia coli	0.00 ± 0.00^{c}	16.50 ± 0.50^{c}	12.50 ± 0.50^{c}

BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves. Values are presented as mean ± SE of duplicates, values in the same column carrying same superscript are not different significantly (p-value < 0.05) according to new duncan's multiple range test

Table 3. Minimum Inhibitory Concentration (MIC) of the plant extracts against the bacterial isolates

Isolate	BW (mg/ml)	BE (mg/ml)	BEA (mg/ml)
Staphylococcus aureus	NT	NT	NT
Staphylococcus aureus	NT	25	NT
Streptococcus pyogenes	NT	NT	NT
Streptococcus pyogenes	NT	NT	50
Streptococcus pneumoniae	NT	NT	NT
Streptococcus pneumoniae	NT	25	NT
Pseudomonas aeruginosa	NT	50	25
Pseudomonas aeruginosa	50	50	100
Haemophilus influenzae	NT	50	NT
Haemophilus influenzae	25	50	100
Klebsiella pneumoniae	NT	NT	50
Klebsiella pneumoniae	NT	50	50
Escherichia coli	NT	100	NT

BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves, NT: not tested

Table 4. Minimum Bactericidal Concentration (MBC) of the plant extracts against the test bacterial isolates

Isolate	BW (mg/ml)	BE (mg/ml)	BEA (mg/ml)
Staphylococcus aureus	NT	NT	NT
Staphylococcus aureus	NT	12.5	NT
Streptococcus pyogenes	NT	NT	NT
Streptococcus pyogenes	NT	NT	25
Streptococcus pneumoniae	NT	NT	NT
Streptococcus pneumoniae	NT	25	NT
Pseudomonas aeruginosa	NT	50	25
Pseudomonas aeruginosa	50	50	100
Haemophilus influenzae	NT	50	NT
Haemophilus influenzae	25	50	100
Klebsiella pneumoniae	NT	NT	50
Klebsiella pneumoniae	NT	25	50
Escherichia coli	NT	100	NT

BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves, NT: not tested

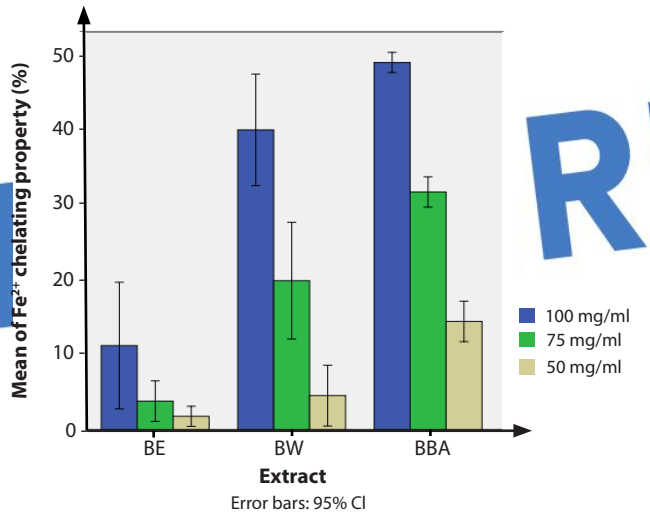

Figure 1. Fe^{2+} chelating (%) property of the plant extracts (BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves)

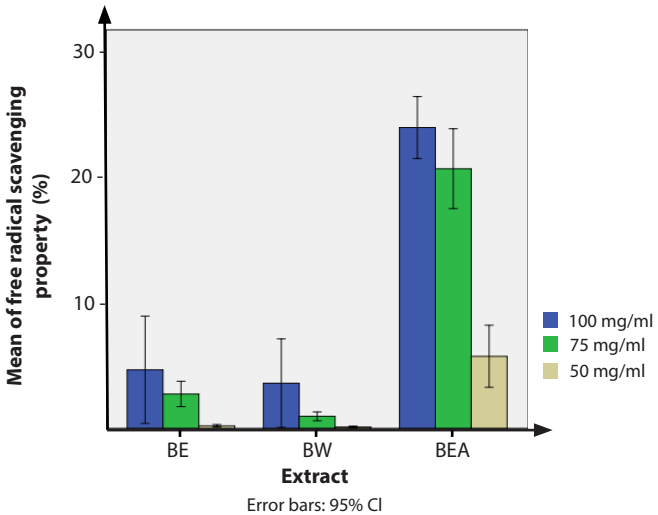

Figure 2. Free radical-scavenging (%) property of the extracts (BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves)

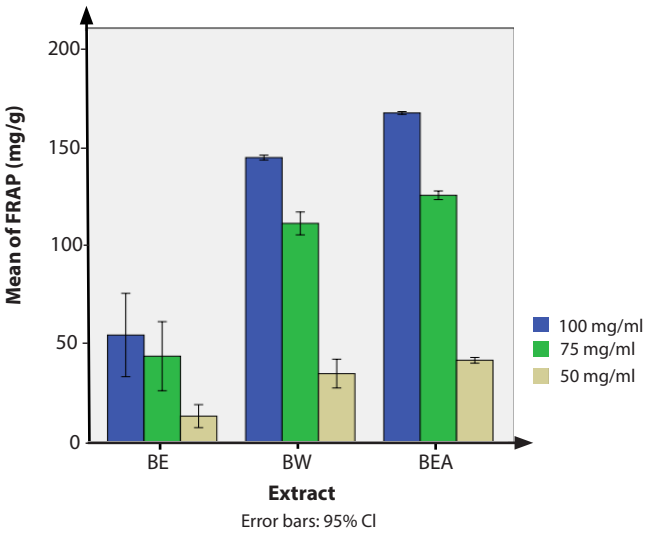

Figure 3. Ferric Reducing/Antioxidant Power (FRAP) (mg/g) of the plant extracts (BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves)

Table 5. Phytochemical (quantitative) constituents of *B. pinnatum* leaves extracts

Phytochemical constituent	BE (mg/g)	BEA (mg/g)	BW (mg/g)
Saponin	18.09 ± 0.27c	13.73 ± 0.27^{b}	10.09 ± 0.27^{c}
Terpenoid	23.28 ± 0.04^{a}	15.81 ± 0.04^{a}	11.56 ± 0.04^{b}
Steroids	7.18 ± 0.02^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
Tannin	4.84 ± 0.01^{e}	1.66 ± 0.01^{d}	2.16 ± 0.01^{d}
Glycosides	21.40 ± 0.05^{b}	13.20 ± 0.05^{c}	17.51 ± 0.05^{a}

BE: ethanol extract of *B. pinnatum* leaves, BW: aqueous extract of *B. pinnatum* leaves, BEA: ethyl acetate extract of *B. pinnatum* leaves. Values are presented as mean ± SE of duplicates, values in the same column carrying same superscript are not different significantly (p-value < 0.05) according to new duncan's multiple range test

DISCUSSION

The extraction of bioactive compounds, or phytochemicals, from medicinal plants is crucial for effective drug development. The percentage yield of the extracts, as shown in Table 1, varied in this study, with the highest percentage yield obtained from the ethanol extract (12.2%) of *B. pinnatum* and the lowest yield obtained from the ethyl acetate extract. This is explained by the fact that the extraction solvents' polarity varied, affecting how soluble the components were in each solvent (Kirmani *et al*., 2024). Also, the percentage yield obtained in each extract further suggests that plants are rich reservoirs of phytochemicals or bioactive compounds (Abubakar *et al*., 2017). Solvent polarity and temperature determine the quantity of phytochemicals that are extracted (Adeleke *et al*., 2007). This study also showed differences in the colors of the extracts obtained, which could result from the different compositions of each extract. The different percentage yield and colors of the extracts corroborate the previous findings where the yield and colors of different extracts varied according to the extraction solvent used (Abubakar *et al*., 2017; Adeleke *et al*., 2007; Kirmani et al., 2024).

The results as shown in Table 2 of the antibacterial activities of *B. pinnatum* leaves extracts showed that the extracts were effective against the selected bacterial pathogens in this study. According to Daniel *et al*. (2020) the methanol and ethyl acetate extracts of *B. pinnatum* leaves have broad-spectrum antibacterial activities. The ethanol extract had the highest activity, while the aqueous extract had the lowest activity against the test isolates. This may be explained by the ethanol extract's greater concentration of bioactive chemicals than the other extracts, which are primarily responsible for the extract's antibacterial properties. Isolation and determination of the individual antibacterial efficacy of each of the bioactive components will further suggest the exact one with the antibacterial potency to be utilized in drug development. The MIC assay in this study revealed the extracts possess in vitro antibacterial activities at varying concentrations. This corroborates with the findings of Ikechukwu *et al*. (2017) and that at different concentrations plant extracts possesses antibacterial properties. The MIC values obtained in this study ranged from 25 mg/ml to 100 mg/ml while the MBC was from 12.5 mg/ml to 100 mg/ml. According to Bosso and Innalegwu (2018) the MIC and MBC of a plant extract and honey indicates their antibacterial efficacy.

In other words, the lower the MIC and MBC values, the higher the potency of the plant.

The findings from this study demonstrated that *B. pinnatum* leaf extracts have strong, dose-dependent antioxidant qualities. The higher the concentration of the extracts, the higher the antioxidant potential of the plant. This corroborates with the findings of (Onoja *et al*., 2018), who reported the antioxidant activities of *B. pinnatum* methanol extract to be concentration dependent. The ethyl acetate extract of the plant has the highest antioxidant properties compared to the other two as reported in this study. This could be attributed to the ability of the solvent to specifically extract bioactive compounds with antioxidant activities which have not been investigated in this study.

The ability of the extracts to serve as reducing agents is mainly due to the presence of reductones which are known to provide antioxidant activities through the breaking of free radical chain as well as donating a hydrogen atom. The excessive production of free radicals such as Reactive Oxygen Species (ROS) are mostly found to be responsible for degenerative diseases such as Parkinson and Alzheimer diseases. According to this study's findings, the extract has the ability to reduce radical chain reactions by acting as an electron donor and reducing power. Investigation of the presence of reductones in the extracts will aid in establishing the antioxidant potential of the plant as a reducing agent.

Table 5 shows the quantity of phytochemical constituents present in each of the plant extracts. The phytochemical constituents that were recorded in this study include saponins, terpenoids, steroids, tannin and glycosides. *B. pinnatum* is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, saponins, tannins, terpenoids, coumarins, bufadienolides, and lipids (Daniel *et al*., 2020; Izunwanne *et al*., 2017). *B. pinnatum* ethanol extract had the highest amount of each of the phytochemicals screened compared to the other two extracts in this study. This was also demonstrated by the highest percentage yield that was recorded in Table 1 by the ethanol extract of the plant. The likely reason is that these bioactive compounds are more soluble in ethanol than the other solvents. According to According to Nawaz *et al*. (2020), the extraction of phytochemicals from plants are usually affected by different factors such as time, temperature, solvent concentration and solvent polarity.

Tannins have been shown to exert antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic and vasodilatory effects (Smeriglio *et al*., 2017). Scavenging free radicals and blocking lipid peroxidation and lipoxygenases are among tannin's antioxidant qualities (Smeriglio *et al*., 2017). Saponins have been reported to possess anti-tumor, antioxidative, antiphotoaging, anti-inflammatory, anticancer (Mbaveng *et al*., 2018; Saleri *et al*., 2017; Yildirım and Kutlu, 2015), antibacterial, antidiabetic, and neuro-protective activities (Nguyen *et al*., 2020). According to Jahangeer *et al*. (2021), the medicinal importance of terpenoids includes antibacterial, antiparasitic, anti-inflammatory, anticancer, and skin protective. Steroids are reported to possess anesthetic, anticancer (Thao *et al*., 2015), anti-asthmatic, anti-hormone (Jovanović-Šanta *et al*., 2015), and anti-inflammatory properties (Lopez *et al*, 2011) cardiovascular agents, and antibiotics. Glycosides show various bioactivities, including antioxidant, anti-inflammatory, antibacterial, antiviral, and antitumor activities (Zhang *et al*., 2022). According to Lawan *et al*. (2023) strong bioactive substances including flavonoids, steroidal glycosides, polyphenols, tannins, and glycosaponins have been shown to be present in *B. pinnatum*, and they are responsible for their antimicrobial, antioxidant, antipyretic, anti-inflammatory, anti-arthritic, anti-allergic, analgesic, antiseptic, sedative, anti depression, wound healing, hepatoprotective, nephroprotective, and urolithic properties. The plant used in this study could possess more bioactive compounds which have not been investigated.

CONCLUSION

The antibacterial activities of *Bryophyllum pinnatum* leaf extracts against some selected pathogens in this study confirm the potency of the leaf for the treatment of infections caused by the test bacterial isolates. The ethanol extract of the leaf showed better activity against the isolates. The antioxidant properties displayed by the extracts of *B. pinnatum* leaves are concentration dependent, and the ethyl acetate extract has better antioxidant potential. The phytochemicals that are present in *B. pinnatum* leaf extracts in this study are saponins, terpenoids, steroids, tannins, and glycosides.

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