RESEARCH ARTICLE

Evaluation of Bioactive Constituents and Antimicrobial Activity of *Bryophyllum pinnatum* Against Bacterial and Fungal Pathogens (Antimicrobial Activity of Phytochemicals Associated with *Bryophyllum pinnatum*)

Leena Das* and V. A. Kamble

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Abstract

The existing study was aimed at evaluating secondary metabolites present in *Bryophyllum. pinnatum* and its antimicrobial activity. The methanolic, ethanolic and chloroform extracts were subjected to phytochemical screening, gas chromatography-mass spectrophotometry (GC-MS), Fourier transforms infrared spectroscopy (FTIR) and antimicrobial investigation. Gas chromatography-mass spectrophotometry (GC-MS) study of the whole plant was performed using a Gas Chromatography /Mass Spectrophotometry Quadra pole Shimadzu model and interpretation was done on the mass spectrum was completed by means of the record of the National Institute of Standard and Technology (NIST) and the FTIR spectrum was noted in a spectrophotometer. Phytochemical screening for plant extracts showed the occurrence of numerous secondary metabolites like alkaloids, cardiac glycosides, flavonoids, phenols, terpenoids, and saponins. Agar well diffusion assay was performed to evaluate the antimicrobial activity of methanol, ethanol and chloroform fractions of *Bryophyllum pinnatum* against fungi and bacteria. Methanol extracts of *B. pinnatum* were found to show the highest inhibition zone against *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans*. Plant extracts were compared with standard drugs, where they showed comparable inhibitory activity. Major compounds identified in GC/MS were dodecanedioic acid bis ester, beta carotene, caryophyllene, vitamin A aldehyde, Kauren-18-ol, acetate, phytol, squalene, 3 carene, alpha-phellandrene and hexadecenoic acid. The present study indicates that phytochemicals present in methanolic, ethanolic and chloroform extract revealed that *B. pinnatum* is appropriate for use in different fields, viz., therapeutic and pharmaceuticals and are of great value in medicinal practice for the treatment of several human ailments. *S. aureus* and *E. coli* were selected since these are the two most common bacterial pathogens and *C. albicans* is the common fungi to cause fungal infections, hence were considered for the study.

Keywords: *Bryophyllum pinnatum*, Phytochemicals, GC-MS, FTIR, Antimicrobial activity, MIC.

Highlights

- *Bryophyllum pinnatum* can be considered as a therapeutic plant.
- The major compounds identified by GC/MS were beta carotene, caryophyllene, phytol, squalene, 3 carene, alpha-phellandrene and hexadecenoic acid.
- Various phytochemical classes, such as terpenoids, saponins, glycosides, alkaloids, and phenols, are present in plant parts.
- The methanolic extract of the plant parts was the most effective among the ethanolic and chloroform extracts.
- Minimum inhibitory concentration was performed.

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Introduction

Medicinal plants include active ingredients acquainted to cure ailment and release from pain or discomfort (Okigbo *et al.*, 2008). The practice of using traditional therapy derived from medicinal and therapeutic parts of plants, mainly in emerging countries, acts as restorative agents for healthiness (Westh *et al.*, 2004). Pharmacopoeia in modern days, however, shows a significant number of drugs derived from plants. Involvement of medicinal plants was done after the occurrence of resistance against standard drugs used for the treatment of human health (Madhu *et al.*, 2016). The hope of medicinal plants increases due to the significant positive activities shown by medicinal tree-derived products (Kala CP., 2005). The therapeutic properties of plants come from the antibacterial, antifungal, antioxidant, and antimicrobial effects of the phytochemical classes present in the plants (Adesokan *et al.,* 2008). According to WHO, medicinal and therapeutic plant parts are the greatest cause of finding valuable remedies. Thus, various activities and properties should be studied for the compounds isolated from plant (Nascimento *et al.*, 2000). However, many plants are yet to be studied in detail to know their medicinal properties. So, it is necessary to study more medicinal plants for their therapeutic properties (Ogundiya *et al.*, 2006).

Department of Microbiology, Adarsha Science, J.B. Arts and Birla, Commerce Mahavidyalaya, Amravati, India.

***Corresponding author:** Leena Das, Department of Microbiology, Adarsha Science, J.B. Arts and Birla, Commerce Mahavidyalaya, Amravati, India., Email: leena18das@gmail.com

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In India, numerous plants have been shown to possess medicinal properties (Sethiya *et al*., 2010). Medicinal plants have numerous secondary metabolites, which play a major role in preventing diseases. For bacterial infections, a wide range of antibiotics of microbial origin and some other chemotherapeutic drugs are commonly used (Othman *et al*., 2019). However, nature has given every living organism a remarkable capacity to fight for survival under unfavorable conditions as proposed by Darwin, hold true for microorganisms too. With the introduction of newer antibiotics in clinical practice, resistance to them also evolves in microorganisms. Since past two decades, there have been many reports indicating the appearance of resistance in bacteria and fungi, not only to single but multiple antibiotics (Ogundiya *et al*., 2006).

Medicinal plants are vast reservoirs of various phytochemicals (Arthur H.R.,1954). The medicinal and therapeutic properties of the plant and plant parts are mainly credited to the phytochemicals present in it. The most important classes of phytochemicals are alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, terpenes, etc., which are present in various plant parts (Njoku *et al*., 2009). Although these classes of phytochemicals are not essential for the plant produced by them, they show a vital role in the existence of plants by protecting the plants through various modes (Kocabas A 2017). The secondary metabolites made by the plants to defend themselves in contrast to various strains, have proved to protect human beings from various diseases (Jan *et al.,* 2021).

Numerous studies have exhibited medicinal and aromatic plants are rich reservoir of bioactive complexes which are highly therapeutic against human pathogens.

It is seen that herbal medicines are safe and available and do not have any side effects when compared with standard drugs. Herbal preparations are more in use nowadays due to the development of the multidrug resistance phenomenon of pathogens (Doughari *et al*., 2009).

Nowadays, bacterial and fungal infections represent an important cause of death worldwide. Threats to antibiotic resistance are increasing nowadays. Therefore, the development and discovery of antimicrobial drugs from natural plant origin for the treatment of microbial infections are of great interest (Chanda *et al*., 2011).

In last few years a number of studies are performed to check the efficiency of plant extracts against bacterial and fungal pathogens. The concern towards the use of traditional medicine nowadays is given much importance for maintaining good health (Ghosh *et al.,* 2015).

Bryophyllum pinnatum (common name – Kalanchoe Pinnata) is one such medicinal plant that belongs to the Crassulacea family. Medicinal plants like *B. pinnatum* possess phytochemical classes such as alkaloids, flavonoids, tannins, saponins and triterpenes (Ojewole, 2005). Our ancestors used leaf juices of these plants for excretory organ stones treatment, headache and inflammation, cancer and wound infections and for treatment of the scalp (Wikipedia).

With all this background, the present study is focused on screening of secondary metabolites and analysis of these bioactive compounds from ethanol, methanol and chloroform extracts of *B. pinnatum* by GC/MS and FTIR profiling is done for identification of compounds. The compounds isolated from *B. pinnatum* through GC/MS analysis were subjected for antimicrobial testing through agar well diffusion study. MIC was done by broth dilution method.

Methodology

Preparation of plant extracts

Whole plant of *B. pinnatum,* including leaves, bark, stem, and flowers, was collected from the Amravati district. The plant parts were cleaned and dried for 7 days and then fine powder was prepared of the whole plant by grinding. About 500 gm of dry powder was extracted with 100 mL of methanol, ethanol and chloroform (80%) by using the soxhlet apparatus. The extracted portion of ethanolic, methanolic and chloroform extract was filtered and a hot air oven was used to evaporate solvents. The residue was stored separately in air containers and preserved in deep freezer.

Qualitative determination of phytochemicals

Phytochemical analysis of an ethanolic, methanolic and chloroform extract of *B. pinnatum* plant was done for determination of bioactive classes such as terpenes, alkaloids, tannins, flavonoids, carbohydrates, proteins, phenols, saponins using standard methods (Yadav *et al*., 2011, Harbone., 1998) as described below.

GC/MS analysis of plants extracts

Gas chromatography analysis of ethanol, methanol and chloroform extract of *B. pinnatum* was performed on instrument GCMS -QP ultra, possessing a capillary column as described in the method by (Hossain *et al*.; 2011). The carrier gas used was helium. Three plant Extracts were dissolved in 1-mL methanol solvent (HPLC) grade and subjected to centrifugation at 3000 rpm for 15 minutes. The supernatant was transferred to a sample tube and from that 1-µL of methanol, ethanol and chloroform extract were injected into GC. The test was performed using an Agilent 6890 gas chromatograph outfitted with a 15 m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness) and a straight deactivated 2 mm direct injector liner. Mass spectra in the range of 30 to 600 were recorded. The identification of compounds was done using NIST library by comparing with mass spectra.

Gas chromatography-mass spectrophotometer analysis was carried out using gas chromatography attached to mass detector for the identification of chemical constituents. Stored databases in a spectrometer were compared with fragmentation patterns of mass spectra.

Identification of compounds

GCMS mass spectrum was interpreted with the help of the record of the National Institute of Standard and Technology.

FTIR

Fourier transform infrared spectrophotometer (FTIR) is used for the identification of various types of functional groups present in compounds. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. Dry powder

extracted from each plant material using various solvents are used for FTIR analysis. 10 mg of dry plant extract powder was crushed with 100 mg of potassium bromide pellet in the sample disc. The crushed portion of each plant extract were laden in FTIR having a scan range of 4000 to 400 cm^{-1}.

Preparation of plant extracts for antimicrobial activity

Whole plant of *B. pinnatum,* including leaves, bark, stem and flowers, were collected from the Amravati district. The plant parts were cleaned and dried for 7 days and then fine powder was prepared of the whole plant by grinding. About 500 gm of dry powder extraction was done with 100 mL of ethanol, methanol and chloroform (80%) by using a soxhlet apparatus. The extracted portion of ethanolic, methanolic and chloroform extract was then filtered and kept in a hot air oven to evaporate the solvents used. The residue was stored separately in air containers and kept in a deep freezer. Plant extracts that showed the presence of different classes of secondary metabolites with help of physical, chemical, and instrumental analysis were screened and later on plates were used to check its antimicrobial efficacy by agar well diffusion assay method and analysis of minimum inhibitory concentration (MIC) done to know its minimum required quantity to inhibit the pathogens.

Collection of Bacterial, fungal samples and antibiotics associated

The isolates include (*Escherichia coli,* NCIM 5347), (*Staphylococcus aureus,* NCIM 5345) and fungal culture (*Candida albicans* NCIM 3674), which were acquired from National Culture of Industrial Microorganisms, Pune and after receiving the cultures, gram staining and biochemical characterization were done. After confirmation through biochemical test, sub-culturing was done and inoculum were prepared for bacterial and fungal culture.

Inoculum standardization

Bacterial cultures matching the turbidity of the 0.5McFarland standards were done. The resulting culture was then used as inoculum for the study used in the well diffusion assay method.

Agar well diffusion method

Agar well diffusion assay method was done according to the standard of the National Committee for Clinical Laboratory Standards (Clinical And Laboratory Standard Institute, 2009) to determine the antibacterial and antifungal activity. Muller Hinton agar and Nutrient agar plates were mopped using sterile cotton swabs impregnated with 24 hours old culture and with a count having 100 cfu/mL of bacteria, and for fungi, 48 hours old culture of 50 cfu/mL culture was used.

Antibiotics such as clindamycin, vancomycin used for bacterial culture and fluconazole used for fungal culture were procured and 1 mg/mL concentration was prepared to check the susceptibility in comparison with the plant extracts.

Four Wells (6 mm diameter and about 2 cm apart) were made with help of a sterile cork borer for *B. pinnatum* extracts. Stock solution of each plant extract were prepared for different agglomeration (10, 5, 2.5, 1.25 mg/mL) in different solvents such as Methanol, Ethanol and chloroform. Plant solvent extracts of about 100 µL of varying concentration were added into the wells and for 2 hours it was kept at room temperature to get diffused. Control experiments were set up to check the growth promotion of the organisms without presence of plant extracts. The plates were incubated for 18 to 24 hours for bacteria at 37°C and for 48 hours at 22°C for fungi. Diameter of zone of inhibition (mm) were measured.

For assessing its comparability with standard drugs, solutions of vancomycin, clindamycin and fluconazole were obtained by dissolving in Muller Hinton broth. Three antibiotics of different range 10, 5, 2.5 and 1.25 all concentrations were prepared in mg/mL were prepared and antimicrobial test was done against pathogens by measuring zone of inhibition. After assessing the antimicrobial action shown by plant extracts, then the plant extracts with different concentrations were used for minimum inhibitory concentration *(MIC).*

Minimum inhibitory concentration determination (MIC)

The fractions that have shown antibacterial and antifungal activity were further assessed for the minimum inhibitory concentration (MIC), technique followed according to (Jennifer, 2001) which is demarcated as the minimal concentration of plant extracts that constrains bacterial and fungal growth. (1 mg/mL) of stock solution was primed by liquefying 100 mg of dried plant extracts each in 5 mL of methanol, ethanol, and chloroform and from this reference 2-fold serial dilution were prepared of different agglomeration of 10, 5, 2.5, 1.25 and 0.625 mg/mL. This concentration of plant derived extracts was utilized for determining minimum inhibitory concentration.

Broth dilution method was used for calculating minimum inhibitory concentration. Briefly, 2 mL of Muller Hinton and nutrient broth were added into 6 test tubes and 0.1 mL of each extract of required concentration which was prepared was added with the nutrient broth and Muller Hinton broth, respectively for bacteria and fungi. After that 0.1 mL of inoculum of bacteria and fungi were added into the test tube containing the extract and the broth, after that all test tubes were properly closed and incubated for 24 hours at 37°C for bacteria and at 22 °C for 48 hr for fungi. Varying visible growths were detected. Minimum Inhibitory Concentration (MIC) was considered as the lowest concentration without the evolution of organisms. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) was done to assess the quantity of drug required to kill bacteria and fungi growth, respectively. For each batch two control tubes were maintained. The two tubes contained extract without inoculum and the other one contains the growth medium and inoculum.

Results

Qualitative analysis of Phytochemicals present in different extracts of *B. pinnatum* is present in Table 1.

Gas Chromatography /Mass Spectrophotometry

The chemical compounds recognized by the Gas Chromatography/Mass Spectrophotometry analysis of the ethanolic, methanolic and chloroform plant extract of *B. pinnatum* was enumerated along with their molecular formula, retention time, and molecular weight. The GC/MS analysis of *B. pinnatum* exposed the occurrence of following compounds

Table 1: Qualitative analysis of Phytochemicals present in different extracts of *Bryophyllum pinnatum*

Plants Extracts	*Alkaloids*	*Terpenoids*	*Coumarin*	*Tannin*	*Flavonoid*	*Phenol*	*Cardiac glycosides*	*saponins*
Ethanol Extract	+	+	-	+	+	+	+	+
Methanol Extract	+	+	-	+	+	+	+	+
Chloroform extract	-	-	-	+	+	-	-	-

Table 2: Compounds isolated from *Bryophyllum pinnatum* using different solvents

S. No.	*Compounds present in methanol portion*	*Compounds present in ethanol portion*	*Compounds present in chloroform portion*
1	Geraniol Tert butyldimethyl silyl ether	3alpha-(trimethylsiloxy)cholest-5- ene	Alpha-phellandrene
2	Tetradecane 22 dimethyl	9,12-octadecadienoic acid	Beta phellandrene
3	Dodecane,222, tetramethyl	Dodecanedioic acid,bis ester	Alpha-phellandrene
4	Caryophyllene	Oleanolic acid	3 Carene
5	Vitamin aldehyde	Beta carotene	Beta phellandrene
6	Glycine,N,methyl ester	Vitamin A aldehyde	Trans pinane
7	Styracitol	Caryophyllene	Squalene
8	Cis inositol	Phytol	Hexadecenoic acid
9	Phytol	Hexestrol	--
10	Isophytol	Rhamnitol – 1-odecyl	-
11	3 Carene	Alpha-phellandrene	-
12	Rhamnitol – 1-odecyl	Hexadecenoic acid	-
13	Octadecanamide	Phenylglycine	-

identified in the methanolic, ethanolic and chloroform extract. The compounds isolated in different extract of *B. pinnatum* are depicted in Table 2.

FTIR analysis

FTIR was used to identify the functional group of various compounds grounded on the wavenumber obtained. The FTIR spectrum of *B. pinnatum* plant extract in the form of KBr pellet is shown in Figs 1-3. The absorption at 3200 to 3700 is due to O-H stretching that are present in the extract. The band observed at 3500 cm^{-1} is attributed for primary amine stretching. The band observed at 2500 to 3300 cm^{-1} is assigned to carboxylic acid stretching. The band founded at 2800-3000 cm^{-1} is because of amine salt N-H stretching. The absorption band observed at 3000-3100 is due to alkene C-H stretching. A notable band 2600 to 2830 cm^{-1} can be assigned to aldehyde C-H stretching. A band observed at 2100 to 2140 cm^{-1} is due to alkyne stretching. A band at 2150 represents ketene C=C=O stretching. A band at 1735 to 1750 is assigned to C=O stretching. A band at 1720 to 1740 is attributed to aldehyde C=O stretching. A band at 1600

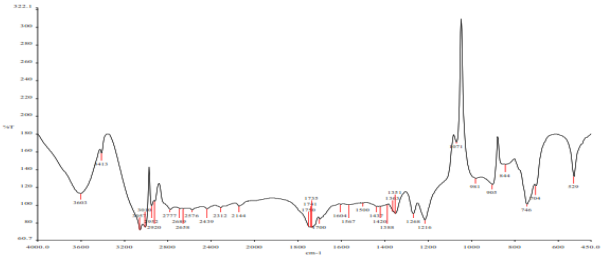

Fig. 1: FTIR graph showing extraction with ethanol solvent and identification of classes from *B. Pinnatum*

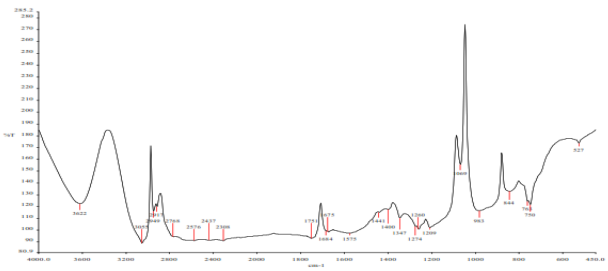

Fig. 2: FTIR graph showing extraction with methanol solvent and identification of classes from *B. Pinnatum*

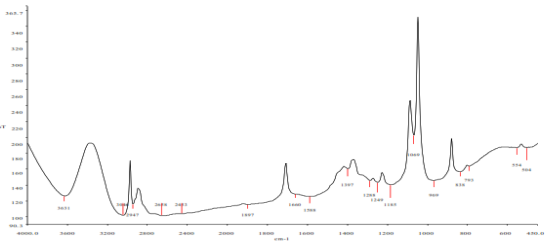

Fig. 3: FTIR graph showing extraction with chloroform solvent and identification of classes from *B. Pinnatum*

to 1678 represents alkene C=C stretching. A band at 1380-1385 represents alkane C-H bending. A band at 1330-1420 is assigned to alcohol O-H bending. A band at 1266 to 1342 represents aromatic amine C-N stretching. A band at 960 to 980 represents alkene C=C bending. A band at 835 to 805 is assigned to aromatic compound C-N stretching. A band at 500 to 730 represents halogen compounds. A band at 665-730 represents alkene with C=C bending. A band with absorption at 490 to 620 represents halogen compound with C-I stretching. Structural features of the *B. pinnatum* extract by FTIR spectrum is depicted in Table 3.

Discussions

Antimicrobial activity and compounds of *B. pinnatum* responsible for therapeutic properties

Based on isolation method, colony and cultural characteristics and biochemical properties, the isolates have been confirmed as *S. aureus E. coli,* and *C. albicans*. Antibiotic sensitivity test against the isolates showed a high sensitivity to vancomycin and clindamycin for experimenting against bacteria and fluconazole for fungi. Antimicrobial action of different plant extracts of *B. pinnatum were* tested against three microorganisms, mentioned in Table 4 along with a standard drug comparison.

In the present study, methanol, ethanol and chloroform portions of B. *pinnatum* plant showed antibacterial and antifungal properties in contradiction of organisms, namely *S. aureus, E. coli*, and *C. albicans*. This antibacterial and antifungal action may be accredited to the occurrence of phytochemical classes such as alkaloids, steroids, glycosides, terpenoids, saponins, flavonoids. This finding is supported by the study of

Table 3: Structural features of the *Bryophyllum pinnatum* extract by FTIR spectrum

Wavenumbers of B. Pinnatum cm -1	*Assignments*
3200- 3700	Alcohol O-H stretching
3500	Primary amine N-H stretching
2500-3300	Carboxylic acid O-H stretching
2800-3000	Amine salt N-H stretching
2600-2830	Aldehyde stretching
2100-2140	Alkyne
2150	Ketene C=C=O stretching
1735-1750	Esters C=O stretching
1720-1740	Aldehyde O=C stretching
1600-1678	Alkene C=C stretching
1380-1385	Alkane C-H bending
1330-1420	Alcohol O-H bending
1266-1342	Aromatic amine C-N stretching
960-980	Alkene C=C bending
835-805	Aromatic compound C-N stretching
500-730	Halogen compound C-CI stretching
490-620	Halogen compound C-I stretching

Table 4: Depicting zone of inhibition (mm) shown by plant extracts using different solvents, Zone of inhibition of plant extracts of *Bryophyllum pinnatum* along with concentration in (milligram/millilitre)

Zone of inhibition of Ethanol extract of Bryophyllum pinnatum along with concentration				
Pathogens	10	5	2.5	1.25
E. coli	19	10	10	-
S. aureus	16	11	11	-
C. albicans	15	12	12	-
Zone of inhibition of Methanol extract of Bryophyllum pinnatum along with concentration				
	10	5	2.5	1.25
E. coli	20	12	8	-
S. aureus	18	12	8	-
C. albicans	18	9	6	-
Zone of inhibition of Chloroform extract of Bryophyllum pinnatum along with concentration				
	10	5	2.5	1.25
E. coli	16	9	8	-
S. aureus	18	15	9	-
C. albicans	18	16	8	-
Clindamycin (antibacterial standard drug)				
	10	5	2.5	1.25
E. coli	20	18	8	4
S. aureus	18	13	9	6
C. albicans	-	-	-	-
Vancomycin (antibacterial standard drug)				
	10	5	2.5	1.25
E. coli	20	18	9	5
S. aureus	18	13	9	6
C. albicans	-	-	-	-
Fluconazole (antifungal standard drug)				
	10	5	2.5	1.25
E. coli	-	-	-	-
S. aureus	-	-	-	-
C. albicans	20	18	16	12

Nwadinigwe and Alfreda (Nwadinigwe *et al*., 2011) who stated that bioactive classes such as glycosides, terpenoids, saponins, flavanoids and alkaloids have been isolated in *B. pinnatum* plant. The main compounds isolated form *B. pinnatum* were Phytol, Caryophyllene, Beta carotene and Carene along with its InfraRed spectrum showing characteristics absorption band for hydroxyl, carbonyl, aromatic, alkene, ether and amine functional group which is similar to the results obtained by Donates and Fred (Okwu *et al.*,2011).

The methanol, ethanol and chloroform portions of plant compared with standard antibiotics (vancomycin, clindamycin and fluconazole) have been reported to have broad spectrum

Table 5: Minimum inhibitory concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) (mg/mL) performance of diverse plant extracts of *Bryophyllum pinnatum* against pathogenic organisms

Microorganisms	*Ethanol extract*	*Ethanol extract*	*Methanol extract*	*Methanol extract*	*Chloroform extract*	*Chloroform extract*
	Minimum Inhibitory Concentration (MIC)	*Minimum bactericidal concentration (MBC)*	*Minimum Inhibitory Concentration (MIC)*	*Minimum bactericidal concentration (MBC)*	*Minimum Inhibitory Concentration (MIC)*	*Minimum bactericidal concentration (MBC)*
E. coli	2.50	5.00	2.50	5.00	5.00	10.00
S. aureus	1.25	5.00	5.00	10.00	5.00	10.00
	Minimum Inhibitory concentration (MIC)	Minimum fungicidal concentration (MFC)	Minimum inhibitory concentration (MIC)	Minimum fungicidal concentration (MFC)	Minimum inhibitory concentration (MIC)	Minimum fungicidal concentration (MFC)
C. albicans	2.50	5.00	2.50	5.00	5.00	10.00

of antimicrobial activity which is in accordance with the study carried out by Akacha (Akacha *et al.*, 2016). Based on these results, vancomycin and clindamycin were used as control for bacterial pathogens and fluconazole for fungi while evaluating the antimicrobial effect of different solvent extracts of *B. pinnatum*.

B. pinnatum methanolic extract showed highest zone of inhibition against *E. coli* and *S. aureus*. The consequences of the antimicrobial effect shows that *B. pinnatum* ethanol portion also has inhibitory properties. This is similar with report of Izundu and Anyamene (Izundu *et al.*, 2021), which stated that stronger antibacterial activity of methanol extracts have been attributed to the potential of the solvent to extract its active compounds from these plant parts like phenolics, saponins and other secondary metabolites which are conveyed to be antimicrobial (Okwu *etal*.,2006). Extracts of decreasing concentration in mg/mL ranging from 10, 5 and 2.5 mg/mL exhibit inhibition as compared to 1.25 mg/mL which showed no inhibition against any organisms. The results obtained showed that zone of inhibition depends on concentration and the results are agreed with the work of Uwimbabazi. (Francine *et al*, 2015).

The ethanol and methanol extracts of *B. pinnatum* showed more inhibition action against three organisms as compared to chloroform extracts. The results were supported by Akkinibosun and Edionwe (Akinnibosun *et al.*, 2015) who concluded that the stronger extraction capacity of methanol and ethanol could have been accountable for the higher antibacterial and antifungal activity. The biological active components in the plant could have been enhanced in the presence of methanol as observed in study conducted by other researchers (Akinnibosun *et al.,* 2015). The antimicrobial prospective of the plant was likely due to the existence of alkaloids and flavonoids in the plant extract (Okwu *et al.*, 2006). The antimicrobial potential of alkaloids and flavonoids are already well recognized (Reddy *etal*. 2007, Cushine *etal,* 2011, Manner *etal*, 2013, Oladejo *et al.*, 2018).

The outcome found from this study showed that the isolates (bacteria and fungi) were inhibited by *B. pinnatum* extracts which is supporting the statement as stated by Donatus (Cushine *et al.*, 2011). The inhibition of fungal pathogens confirms the traditional healing privileges for use of this plant in the dealing of fungal pathogens. These conclusions reinforced the usage of *B. pinnatum* in ethno medicine in Africa for the treatment of ulcers, burns and boils (Okoli *et al.*, 2001).

In this study, as depicted in Table 5, Minimum inhibitory concentration of ethanol and methanol extract extended from 0.625 mg/mL to 10 mg/mL against bacteria and fungi where the minimum inhibitory concentration required was 1.25 mg/mL against bacteria which is much less than the one stated in the study performed by Patrick and Okpoho (Okpoho *et al.*, 2019), where it was indicated that the amount required for MIC was 25 mg/mL. In this study minimum inhibitory concentration was found to be 2.50 mg/mL in case of fungi. Minimum Bactericidal concentration is 5.00 mg/mL in case of ethanol extract and 10.00 mg/mL in case methanol extract in this study. In this work *S. aureus* showed the minimum inhibitory concentration which is somewhat alike to the work of Alfreda and Nwadinigwe (Nwadinigwe *et al.*, 2011).

Conclusion

The plant extract of *B. pinnatum* showed noteworthy inhibitions of clinically significant microorganisms that contain active phytochemicals such as alkaloids, glycosides, saponins, flavonoids and phenols. The compounds investigated through GC/MS were responsible for their antimicrobial properties. Additionally, the methanolic plant extract was the most potent against three organisms and the ethanolic extract showed the minimum inhibitory concentration against *S. aureus*. . Different plant extracts were also compared with standard drugs, where it depict that novel compounds isolated from *B. pinnatum* are of comparable therapeutic agents compared to antibiotics which were used for the study. Hence from the above study, it can be concluded that *B. pinnatum* holds therapeutic properties to produce novel drugs. It is seen that *B. pinnatum* contains various bioactive compounds which are responsible for antimicrobial properties. However, further studies will be undertaken to ascertain the synergistic role of plant extracts of *B. pinnatum* along with the antibiotics used in this study.

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Author's contribution

LD, VK designed all the experiments and LD performed testing of all parameters. LD procured all the necessary materials. LD drafted the manuscript and VK reviewed the manuscript.

Conflict of interest

None

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