EUROPEAN ACADEMIC RESEARCH
Vol. X, Issue 12/ March 2023

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Determination of Phytochemical Components and the Antimicrobial Potency of *Bryophyllum pinnatum* Leaf Extracts against Some Clinical Bacteria and Fungi Isolates

KABIRU HARUNA IBRAHIM
LAWAN SANI AHMAD
MUHAMMAD SULAIMAN
Department of Biological Sciences, Kano State College of Education and Preliminary Studies
MANNIRU ALI ABULLAHI
Department of Chemistry, Kano State College of Education and Preliminary Studies

Abstract

The Bryophyllum pinnatum plant is one of the many medicinal plants that traditional healers use to treat a variety of illness. This study was aimed to identify the ideal phytochemical components and antimicrobial potency of B. pinnatum plant leaf extracts. Seven different extraction solvents of increasing polarity were used to assess the phytochemical properties and biotherapeutic potential of the plant. The fresh leaves were collected from the Garden of Plant Biology Department of Bayero University, Kano. Following the normal and standard procedure, the leaves were air dried and ground into powder and used for extraction, to determine the presence of secondary metabolites. Then follows by antimicrobial efficacy with different concentrations (25, 50, 75 and 100 mg/ml) of the extracts. The microbial strains were collected from Murtala Muhammad General Hospital, Kano. Nine microbes were tested using agar disc diffusion method and commercially prepared antibiotics were used as positive control. The antimicrobial potency of each extract concentration was assessed by measuring the zone of inhibitions against the test organisms. Various phytochemicals were presents, according to the data, the highest quantities of alkaloids, flavonoids, saponins, tannins and terpenoids as well as moderate concentration of phenolic were found in the methanol extract. When employing petroleum ether, resins were discovered in excess quantity. The outcomes also revealed the average zone of inhibitions for different plant extract at various doses. The findings showed that, when compared to other extracts, the ethyl acetate and methanol plant extract of B. pinnatum have higher antimicrobial potency, with methanol having the strongest antimicrobial effect (21.4±0.2 and 24.4±0.2) against E. coli isolate at 75 and 100 mg/ml respectively. The antimicrobial potency of other extracts was moderate to weak. The test organism's susceptibility varied, E. coli was the most susceptible of the test organisms, whereas A. niger and C. albicans showed the least. The overall findings of this study as a whole indicated that alternative solvents may be required for a better extraction of antimicrobial components from particular medicinal plants.

Keywords: Bryophyllum pinnatum leaves, phytochemicals and antimicrobial potency

1. INTRODUCTION

In recent times, morbidity and mortality have been caused by the unavailability and high cost of new generations antibiotics with short period of effectiveness (William, 2000). Findings compounds from alternative sources that have proven antimicrobial action is therefore necessary. As a result, researchers have been looking for more potent antimicrobial agents among material with plant origin in an effort to find potentially

helpful active ingredients may be used as a source and template for the synthesis of novel antimicrobial medications (Pretorious *et al*., 2003, Moreillion, *et al*., 2005). For these reasons, it's crucial to search for new medications that are also readily effective, available, inexpensive and extremely acceptable. Roughly 80% of the world's population relies on herbal medicine for basic health care and plants have formed the foundation of a powerful traditional medical system that has helped to meet the demand for the synthesis of new drugs (Ezegu, *et al*., 2020). More specifically, according to Anjoo and Ajay, (2009) herbal medicine is the oldest form of medical care that has ever been used by humans. Additionally, more than 50% of all current medications are made from natural ingredients, which is significant for the pharmaceutical industry's drug development.

The World Health Organization (WHO) defines medicinal plants as those with one or more of parts contain compounds that can be utilized therapeutically or as a starting point for the production of effective medications. However, it has been reported that medicinal plants are widely employed for the treatment of a number of ailments (Grover, *et al*., 2002). To avoid mistaken destruction of plants, which could serve as a spring board for the development of potent medications, it is necessary to examine their various therapeutic potentials. According to numerous studies on medicinal plants and herbal drug synthesis the bioactive components of medicinal plant found in the leaves, roots, stem and other plant parts. These bioactive compounds popularly referred to as phytochemicals, include terpenes, alkaloids, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraquinones (Iwu, 1993 and Imaobong, *et al.,* 2020). Many plants produce secondary metabolites with antioxidant, antibacterial, antifungal and other biological potentials (Mehrorosh, *et al*., 2014). According to previous studies, approximately 80% of the world's population still relies on traditional medicines for their primary health care, the majority of which uses plant extracts (Sandhya, *et al*., 2006). In the past few years, have seen a rise in the demand for natural substances like plant-rich antimicrobials due to concerns about the safety of synthetic antimicrobial medications (Imaobong, *et al.,* 2020). This is due to report that herbal medicines, especially when compared with synthetic medications, are believe to be side effects-free, accessible, inexpensive and safe (Britton, *et al*., 2002 and Hussain, *et al*., 2010). One of such plants used to treat variety of diseases brought on by pathogenic organisms is *Bryophyllum pinnatum*. *B. pinnatum* plants are in the Crassulaceae family and the common names include life plant, African never die, love plant, miracle leaf, and Canterbury bells, are also known "Shuka halinka or Karan massalaci" in Hausa, a northern Nigerian tribe (Mudi and Ibrahim, 2008). Tropical Africa, America, Hawaii, India, China, Australia, and Madagascar all have significant populations of it (Afzal, *et al*., 2013). *B. pinnatum* is a succulent, 50 – 200 cm tall and about 3.2 cm wide plant that can reproduces vegetatively from leaf bulbils and through seed (Imaobong, *et al.,* 2020). To treat anti-inflammatory, antipyretic, antimicrobial, antioxidant, antitumor, antidiabetic, anti-ulcer, antiseptic, hypocholesterolemic, and cough suppressant conditions, however, the leaves and leaf juice have been employed (Ali, *et al*., 2011). Nonetheless the plant is rich in valuable compounds including polyphenols, tannins, glycosaponins, flavonoids, steroidal glycosides and many other crucial chemical components that are responsible for its anti-oxidant, anti-pyretic, anti-inflammatory, anti-arthritic, anti-allergic, analgesic, antiseptic, sedative, anti-depression, wound healing, hepatoprotective, nephroprotective, tocolysis, urolithic, anti-psychotic, muscle relaxant, anti-protozoal,

anti-microbial and anti-diabetic properties. Moreover, the herb is good source of carbohydrates, proteins, amino acids, lipids, vitamins and mineral elements such as Na, Ca, K, P, Mg, Mn, Fe, Zn. The presence of zinc in the plants may indicate that it can be useful in managing diabetes, which is brought on by ineffective response to insulin. Despite the fact that many aspects of the herb have already explored, it is still required to conduct additional in-depth research of the herb to prove it therapeutic value and evaluate the justification for its usage in traditional medicines.

Therefore, the current study aims to examine this valuable medicinal plant based on is local uses as a treatment of infectious diseases in order to ascertain the phytochemical compositions of the plant and to conduct an antimicrobial activity test to see whether the growth of the bacteria and fungi known to be the causative agents of many infectious diseases could be suppressed.

2. METHODOLOGY

Study Design

An in-vitro experimental study of phytochemical compounds and antimicrobial activity of *B. pinnatum* leaves extract was carried out using seven solvents of increasing polarity and agar well disc diffusion method. In all assays, positive and negative controls were used to assess the potency of the leaf extracts.

Collection and Processing of Plant Material

Fresh leaves of *B. pinnatum* were hand-picked from the Garden of Plant Biology Department of Bayero University, Kano. Plant species was identified and authenticated by a Plant Biology Staff. A paper bag was used to transfer the sample leaves to the lab, and to get rid of the contaminants and other extraneous matter, the leaves were first washed three times with tap water and then rinsed twice with distilled water. In order to prevent photolysis and heat degradation of metabolites, the cleaned plant material were semi ground before being air dried for three days at room temperature in the shade. We used mortar and pestle to ground the dried leaves into powder. Following that, the powdered leaves put through a sieve using a No. 40 sieve and kept in an air tight container until extraction.

Extracts Preparation

The standard method was used to prepare the *B. pinnatum* plant leaf extracts with a slight modification. Seven extracting solvents of increasing polarity were used to prepare the extracts. The extraction was carried out independently. To make 10% in 500 ml sterilized screw cap bottles, 20 g of dried powdered leaves were suspended in 200 ml of the appropriate solvent. To enable the extraction of the active chemicals, the suspensions were placed at 35℃ on an orbital shaker at 120 rpm for 48 hours. The suspensions were passed through a white man (No. 1) filter paper filtered before being evaporated at 40℃ (Yamato RE 801, Japan) under a rotary vacuum to eliminate excess solvents. The extracts were subjected for phytochemical analysis, to determine the presence of secondary metabolites.

Phytochemical Screening

To determine the presence of secondary metabolites in the leaf extracts of *B. pinnatum*, qualitative phytochemical analysis was performed. The phytochemical components of

plants materials were identified using a standard screening approach as per (Kumar, *et al*., 2009, Yusha'u, *et al*., 2009 and Reena, *et al*., 2010) descriptions.

Test for Anthraquinones
Five milliliters (5ml) of the leaf extracts were hydrolyzed with dilute H_2SO_4, extracted with benzene, and then given 1 ml of dilute ammonia. The presence of anthraquinone was shown by the rose pink colour.

Test for Alkaloids
In a test tube, 3 drops of dragendrop were applied to 0.1 ml of the filtered extract. An orange-red precipitate with turbidity was observed, that indicates the presence of alkaloids.

Test for Flavonoids
Five drops of concentrated HCl and 1.5 ml of methanol solution were added to 4 ml of the extract sample. A pinkish tomato red color was observed, that indicated the presence of flavonoids.

Test for Glycosides
In separate test tubes, 10 ml of 50% H_2SO_4 was added to 1 ml of the filtrate extract. The mixture was then heated for 15 minutes before 10 ml of the Fehling's solution was added and the mixture was boiled. A brick red precipitate was observed.

Test for Phenols
A few drops of ferric chloride ($FeCl_3$) solution were also added to the test tube containing the extract sample, along with 2 ml of ethanol. The existence of phenol was confirmed when the color of the resulting combination changed to a deep blue color.

Test for Reducing Sugar
In a test tube, 1 ml of the extract solution was added, followed by 2.0 ml of distilled water, Fehling's solution (A+B), and mixture was warmed at 40℃. At the bottom of the test tube, a brick red precipitate was seen.

Test for Resins
To each of the leaf extracts, 2 ml of acetic acid anhydride and a few drops of H_2SO_4 were added. When violet coloring forms, resins are present, as evidence by the observations.

Test for Saponins
After adding 2 ml of distilled water and vigorously shaking, 2 ml of the leaf extract sample was added to test tubes. There was a frothing seen, that indicates the presence of saponins.

Test for Tannins
A few drops of 1% ferric chloride ($FeCl_3$) solution were added to 2 ml of the extract. There was a blue-black color present. Once more, using a dropper, a few drops of 10% ferric Chloride ($FeCl_3$) were added to each 2 ml of leaf extract. The presence of tannins was suggested by the strong bluish color.

Test for Terpenoids

50 ml of ethanol were macerated with 1g of the leaf sample. The solution was filtered. A test tube containing 2.5 ml of filtrate was pipetted with 2.5 ml of a 5% aqueous phosphomolybdic acid solution, 2.5 ml of concentrated H_2SO_4 was then progressively added, the mixture was stirred, and the tube was allowed to stand for 30 minutes. Ethanol was used to dilute the solution to 12.5 ml and absorbance at 700nm was measured.

Sources of Microorganisms

Clinical isolates of various microorganisms were obtained from the Medical Pathology Murtala Muhammad Specialist Hospital, Kano, Nigeria. Gram reactions and biochemical tests were used to validate the isolates and identify the species according to accepted microbiology procedure. Mueller Hinton Agar medium (Oxoid UK) was prepared in accordance with the manufacturer's instructions, autoclave and distributed to petri-dishes plates, the medium was used to keep the organisms alive. Each time, pure culture that had been stored for 24 hours was used. The isolates were employed as test organisms for antimicrobial assays. Prior to usage sterility of the set plates was verified by an overnight incubation.

Antimicrobial Assays

The antimicrobial assay was performed as described by (Daoud, *et al*., 2015). Agar well diffusion method was employed to screen the antibacterial and antifungal potencies of different extraction solvents. Different concentrations (100, 75, 50 and 25 mg/ml) of *B. pinnatum* were prepared by two fold serial dilutions. Using a sterile cork hole borer, four wells of 6 mm diameter were made on the surface of each agar plate. The plates were inoculated with the test organisms. The wells of the already seeded agar plates were filled with appropriate extracts at varying concentrations. DMSO at a concentration of 10%, and commercially prepared antibiotics (ampicillin, ciprofloxacin and amphotericin B) were used as the negative and positive controls, respectively. The plates were incubated at 37℃ for 24 hours after spending 30 minutes in the refrigerator. All assays were performed in triplicates. The diameter of the zone of inhibitions was measured using a sliding digital micro caliper, the antimicrobial potency of each test extract concentration was evaluated.

Analysis of Data

The results of the antimicrobial potency were analyzed, and the measurement's means and standard deviations were utilized to display the results of the zone of inhibitions. The difference among the means values was evaluated using one way analysis of variance (ANOVA) statistical package for social sciences (SPSS Version 20 Chicago, IL, USA), and then followed by Least Significant Difference (LSD).

3. RESULT

The present study was conducted to identify the phytochemical compounds and antimicrobial activity of the leaf extracts of *B. pinnatum* using various extracting solvents. The results of the phytochemical screening obtained revealed the presence of different secondary metabolites in the leaf extracts of *B. pinnatum* plant. The results shows that all the extracts formed using seven different extracting solvents, *B.*

pinnatum leaves contains phytochemical compounds, like alkaloids, anthraquinones, anthocyanins, flavonoids, glycosides, phenolics, saponins, resins, reducing sugar, tannins and terpenoids (Table 1). Among all the secondary metabolites identified, alkaloids, flavonoids, tannins and terpenoids were found in excess in the extract made by using methanol. However, resins were obtained in high concentration only in the extracts of petroleum ether. But anthocyanin and anthraquinone were found absent in the extract made by using acetone, chloroform and dichloro methane. This indicated that the present used solvents were not able to extract the compounds out from the leaf of *B. pinnatum* or may be due to poor solubility of these phytochemicals in acetone, chloroform and dichloro methane respectively. This also signifies the inefficiency of using acetone, chloroform and dichloro methane as phytochemical extraction solvents especially on *B. pinnatum*. However, it has been reported that different solvents have the capacity to extract varying phytoconstituents depending on their solubility or polarity in the solvents (Loly, *et al*., 2011). This discrepancy might be explained by the fact that extractable bioactive components are more soluble in methanol than in other solvents. The variations in the yields of extracts could be attributes to the difference in solvents polarities which plays a vital role in increasing the solubility of phytochemical compounds (Samir, *et al*., 2017).

However, the antibacterial and antifungal effects of the various extract concentrations (25, 50, 75 and 100 mg/ml) were assessed in this investigation. The disc diffusion method was employed to nine microorganisms including two fungi and seven bacteria (Gram positive (+) and Gram negative (−) bacteria). The antimicrobial potency of all the extracts concentrations was determined by measuring the zone of inhibition against the test organisms. The zone of inhibition as determined varied with the solvents used for extraction, the concentrations and the test organisms (Table 2, 3, 4 and 5). The result shows the mean zone of inhibition for the different plant extract at 25 mg/ml concentration, the result revealed that *B. pinnatum* plant extracts of ethyl acetate and methanol have more antimicrobial action compared to other extracts, with ethyl acetate having the highest antimicrobial activity of 16±0.2 mm against *E. coli* isolates. The chloroform extract demonstrated efficacy against *E. coli, K. pneumonia, P. aeruginosa* and *S. aureus* but no other isolates. Dichloro methane had no effects on any of the species that were examined. While there was no inhibition against other isolates, the petroleum ether extract exhibited slight action against three of the tested organisms.

However, the most significant antimicrobial effectiveness was also demonstrated by methanol extracts against *E. coli*, the effect was more pronounced using high concentration, with zone of inhibitions of 21.4±2 mm at 75 mg/ml and 24.4±2 mm at 100 mg/ml respectively. This indicated that the methanol extract was the most active among the extraction solvents because it demonstrated antimicrobial activity against all the tested species. The increased solubility of extractable bioactive components found in methanol than other solvents may be responsible for the difference in potency. This outcome is consistent with the findings of Ofjokansie, *et al*., (2005) that described strong action of methanol extract of *B. pinnatum* against some gram positive bacteria. The result, however, is reconciled that of Imaobaong, *et al*., (2020) who observed that *P. aeruginosa* was the test organisms with the highest susceptibility and that plant leaf extract in methanol was most efficient against it. This signified that, the ability of solvent to extract some of the bioactive compounds may be the cause of the methanolic extract's antimicrobial activity on the tested organisms. And this explains

why the synergy utilizing methanol as the extracting medium has the highest antimicrobial activity. As a result the extract demonstrated potential antimicrobial potency against the test organisms and may be used to treat infectious diseases brought on by such pathogens. Other extracts had moderate to weak antimicrobial effects. This might be because an active ingredients are absent or because the solvents can't completely dissolve and extract all of the active chemicals. The chloroform and diethyl ether extract had the least impact on the test organisms. However, the majority of the test organisms were dichloromethane resistant. This may be as a result of the solvent's inability to extract most of the bioactive elements from *B. pinnatum* leaf. The result contradicts with the findings of Akinibosun and Edionwe, (2015), who reported that acetone extract was found to be the least effective compared to other extracting solvents.

Eschericia coli, however, had the highest susceptibility among the test organisms, whilst *Candida albicans* and Aspergilus displayed the lowest susceptibility. The potency of the various *B. pinnatum* extract concentrations were $25 < 50 < 75 < 100$ mg/ml. The extracts synergistic effects were concentration dependent. Thus increasing the concentration of the plant extracts will result in higher activities against test organisms, hence, the result of the current study suggested that a concentration increase could enhance the impact of plant on microorganisms. This has implications for pharmacology when formulating and using plant extracts to make antimicrobial medications. However, the effects of the *B. pinnatum* leaf extracts on the test organisms varied, and it has been hypothesized that this difference in activity may be due to variations in the phytochemical substances found in the extracts. The chemical composition of plant extract, which varies depending on the type of solvent employed in the extraction process, determines the antimicrobial activity of the used plant leaf extracts. And this research work revealed that certain medicinal plants may need different solvents for better extraction of antimicrobial components. The optimum extraction solvents, however, were determined by the current study and can be used to extract the maximum quantity of phytoconstituents from *B. pinnatum* plant leaves. The findings revealed the following trend in the various extracts potency, methanol < ethyl acetate < petroleum ether < acetone < chloroform < diethyl ether < dichloro methane.

However, the *B. pinnatum* plant's traditional usage for treating infectious diseases is possibly explained by its antimicrobial qualities. The traditional usage of the extract made from fresh leaves of this plant to fight antimicrobial infections is justified by this research work. In terms of new drugs discoveries derived from plant sources, the overall results of this study are quite recommending. However, practitioners of both conventional and alternative medicine should be encouraged to employ plant extracts in the treatment of some infectious diseases brought on by microorganisms, while the pharmaceutical industries should continue incorporate plant extracts into the production of antibiotics or drugs.

Table 1. Phytochemical Constituents of the *B. pinnatum* Leaf Extracts Using Different Extracting Solvents

S/N	Phytochemicals	Acetone	Chloroform	Dichloro methane	Diethyl ether	Ethyl acetate	Methanol	Petroleum ether
1	Alkaloids	+	++	+	+	+	+++	++
2	Anthocyanins	ND	−	−	−	−	−	−
3	Anthroquinones	ND	−	−	+	−	+	+
4	Flavonoids	+	++	−	+	++	+++	−
5	Glycosides	+	++	−	+	++	+	−
6	Phenolics	+	+	−	+	+	++	+

7	Reducing sugar	+	+	+	+	+	+	+ +
8	Resins	–	–	+	–	–	+	+ + +
9	Saponins	+	+	–	+	+	+	–
10	Tannins	+	–	–	+	+ +	+ + +	–
11	Terpenoids	ND	+ +	+	+	+ +	+ + +	+ +

– Absent, + slightly present, + + moderately present, + + + highly present, ND Not detected.

Table 2. Mean Zone of Inhibitions (in mm) for the Different Leaf Extracts of *B. pinnatum* at 25 mg/ml Concentration.

Microbial isolates	Acetone	Chloroform	Dichloro methane	Diethyl ether	Ethyl acetate	Methanol	Petroleum ether
E. coli	4.1±1	5±1.2	NI	NI	16.1±0.2	4±0.2	5±1.5
K. pneumonia	2.1±1	7±0.1	NI	NI	11±0.2	2.1±1	NI
P. aeruginosa	NI	10±0.3	NI	NI	NI	5.1±1	NI
S. aureus	5.2±1	5±1	NI	2±1.2	11±0.5	4±1.3	6±1.1
S. typhi	6.1±1	NI	NI	3±1.2	9±0.4	5.1±1	4±1.3
S. dysenteriae	4.2±1	NI	NI	NI	NI	2.1±1	NI
A. niger	NI	NI	NI	NI	NI	4±0.2	NI
C. albicans	NI	NI	NI	NI	7.1±0.5	1.4±1.	NI

NI = No inhibition, values are means inhibition zone (mm) ± SD of three replicates.

Table 3. Mean Zone of Inhibitions (in mm) for the Different Leaf Extracts of *B. pinnatum* at 50mg/ml Concentration.

Microbial isolates	Acetone	Chloroform	Dichloro methane	Diethyl ether	Ethyl acetate	Methanol	Petroleum ether
E. coli	8±1.3	7±0.4	NI	2.1±1	10±0.6	9.3±1	7±1.4
K. pneumonia	5±1.4	4±1.2	NI	NI	13±1.2	3.1±2	NI
P. aeruginosa	6±1.2	11±0.2	NI	NI	NI	5.2±1	4±0.5
S. aureus	7±1.5	12±1	4.1±1.3	6±0.4	10±0.12	6.1±1	6±1.3
S. typhi	5±1.3	6±0.2	NI	8.1±1.4	4.1±0.2	7.1±3	6±0.5
S. dysenteriae	4±1.4	3±1.0	NI	NI	NI	6.3±1.2	NI
A. niger	NI	NI	NI	NI	NI	4.1±1	1.5±1.0
C. albicans	NI	2±0.1	NI	NI	11±0.4	2.1±1	NI

NI = No inhibition, values are means inhibition zone (mm) ± SD of three replicates.

Table 4. Mean Zone of Inhibitions (in mm) for the Different Leaf Extracts of *B. pinnatum* at 75mg/ml Concentration.

Microbial isolates	Acetone	Chloroform	Dichloro methane	Diethyl ether	Ethyl acetate	Methanol	Petroleum ether
E. coli	8±1	9±0.2	4.1±1.2	6±1.5	11±1.2	21.4±2	9.3±0.5
K. pneumonia	5±1	6±1.0	3±0.3	NI	14±0.3	8.3±1	5.7±1.0
P. aeruginosa	7±1	12±1.2	NI	NI	NI	6.4±1.3	7.0±2.0
S. aureus	8±1	13±1.3	6±1.2	7±1.6	12±0.2	10.2±1	8.7±1.5
S. typhi	7.1±1	8±0.4	NI	10±0.2	10±1.3	9.2±1.2	7±1.2
S. dysenteriae	6±1	5±1.0	NI	3±0.3	8±0.02	8±1.4	5.7±1.6
A. niger	4.1±1	NI	NI	NI	NI	9.3±1.2	2.0±1.5
C. albicans	5±1	4±1.4	NI	NI	15±0.23	9.2±1.4	NI

NI = No inhibition, values are means inhibition zone (mm) ± SD of three replicates

Table 5. Mean Zone of Inhibitions (in mm) for the Different Leaf Extracts of *B. pinnatum* at 100mg/mm Concentration.

Microbial isolates	Acetone	Chloroform	Dichloro methane	Diethyl ether	Ethyl acetate	Methanol	Petroleum ether
E. coli	12±1	9±1.4	7±1.0	8±1.2	14±0.02	24.4±2	10±1.5
K. pneumonia	10±2	8±0.3	6±1.2	5±0.4	18±0.2	12.3±1	8.7±1.2
P. aeruginosa	9.1±1	14±1.4	NI	NI	NI	13.4±1.3	13±1.5
S. aureus	8.2±1	13±1.5	10±1.2	9±1.3	14±1.02	10.2±1	8.9±1.4
S. typhi	12.1±1	9±1.2	NI	11±1.4	NI	9.2±1.3	9.7±1.5
S. dysenteriae	11±1	6±1.3	4.1±1.2	5±1.2	18±0.52	15±1.5	10±1.3
A. niger	9.3±1.2	2±1.0	NI	2±0.3	NI	12±1	8±1.5
C. albicans	9.2±1.4	7±1.4	NI	NI	17±0.12	10±1.2	NI

NI = No inhibition, values are means inhibition zone (mm) ± SD of three replicates.

4. CONCLUSION

It has been determined that all of the extracts made from *B. pinnatum* leaves using seven different extracting solvents contain phytochemical compounds. Among all the secondary metabolites identified, alkaloids, flavonoids, tannins and terpenoids were found in excess in the extract made by using methanol. *Eschericia coli*, however, had the highest susceptibility among the test organisms, whilst *Candida albicans* and *Aspergilus* displayed the lowest susceptibility. The potency of the various *B. pinnatum* extract concentrations were $25 < 50 < 75 < 100$ mg/ml. However, the presence of various bioactive compounds made the test organisms susceptible, which resulted in the differential antimicrobial potency of the *B. pinnatum* leaf extracts against different bacteria and fungi. And this research work found that some medicinal plants might require alternative solvents for a better extraction of antimicrobial components, further study is needed to confirm this.

REFERENCES

1. Afzal, M., Kazmi, I., and Anwar F. 2013. Antineoplastic Potential of Bryophyllum pinnatum Lam. On chemically induced hepatocarcinogenesis in rats pharmacogonesis research. 5(4)247. https//doi.org/10.4103/0974.8490.118811
2. Akinnibosun, H and Edionwe, O., 2015. Evaluation of the Phytochemical and Antimcrobial Potential of the leaf extracts of B. pinnatum, (L) Citrus aurantifolia S.W and their synergy. *Journal of Appl. Sci Environ Manage.* Vol. 19(4) 611-619.
3. Ali, N., Shah, S.W., Shah, I., Ahmed, Ghias, M., Khan, I. 2011. Cytotoxic and anthelmintic Crude saponins isolated from Achillea wilhelmsil. C. kochi and Tencriums tocksianum. BMC Complementary and alternative medicine. Dec 1., 11(1):106. https://doi.org/10.1186/1472-6882-11-106
4. Anjoo, K., and Ajay, K.S., 2009. Bryophyllum pinnatum (Lam.) Kurz: Phytochemical and Pharmocological Profile; A Review of Phacog Rev. Vol., 3, Isue 6, 364-374 Available on Line: www.phco-grev.com.
5. Britton, R.S., Leicester, K.L., Bacon, B.R. 2002. Toxicity and Chelation therapy, *international Journal of hematology*, 1; 76(3):219-28. https//doi.org/10.1007/BFO 2982791.
6. Daoud, A., Malika, D., Bakari, S., Hfeidh, N., Mnafgui, K., Kadri, A. *et al*., (2015). Aseesment of Polyphenol Composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. *Arab, j. Chem. (inpress). 10.1016 /J. arabjc.* 2015.07.014
7. Ezengu, C.U 2020. Antidiabetic and Antioxidant of *Bryophyllum pinnatum* in Alloxan Induced Diabetic Rats
8. Gill, L.S., (1993). The ethnomedical uses of plants in Nigeria: Benin City, Edo State: Published by University of Benin Press.
9. Gingsburg, H., Deharo, E., 2011. A call for Using natural compounds in the development new antimals antimicrobial treatments- an introduction malaria J. 10:51.
10. Grover, J.K., Yudau, S., and Vats, V. 2002. Medicinal Plants of India with Antidiabetic *Potential Journal of Ethnopharmacol*, 81(1): 81-100
11. Hussain, J., Ullah, R., Rahman, N., Khan, A.L,Muhammad, Z, Husaain F.U, Anwar, S., 2010. Endogenous transitional metal and proximate analysis of selected medicinal plants. Research 4; 4(3): 267-70.
12. Imaobong, E. D., Ekemini, I.A, Edidiong, C. Utum 2020. Phytochemical Evoluation, Antioxidant and Antimicrobial Activities of Various Extracts from *Bryophyllum pinnatum*. *Nepal J Biotechnol*; 8(1):17-28.
13. Isam, E., Fahmi, A.M., and Suad A., 2016. Phytochemical Screening and Bilogical Activity of *Balanites aegyptiaca* Stem bark. *Journal of Chemical and Pharmaceutical Research*, 8(4): 489-498.
14. Iwu, M.M., 1993. Handbook of African Medicinal Plants CRS Press.
15. Latif, A. Ashiq, K., Qayyum, M., Ashiq, S. Ali, E. and Anwer, I., 2019. Phytochemical Profile of the Medicinal Herbs Bryophyllum pinnatum: *The Journal of Animal & Plant Sciences.* 29(6) Page: 1528-1534
16. Loly Elsir, A.H., Hasnah, M.S., Sakina, M.A., Waleed, S.K and Siddiq, I.A 2011. In-vitro Antimicrobial activities of chloroform, hexane and ethanolic extracts of Citrullus lanatus. *Journal of Medicinal Plant Research* Vol. 5(8) pp 1336-1344.
17. Mudi, S.Y and Ibrahim, H 2008. Activit of B. pinnatum S. Kurz Extracts on Respiratory Tract Pathogenic Bacteria. *Bayero Journal of Pure and Applied Sciences*, 1(1): 43-48.
18. Kumar, A., Tievarasan, R., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanbhan, N. and Krishnan, M.RV. 2009. Phytochemicals investigation on a Tropical plant, Syzygium cumini from Kattuppalayan, Erode District, Tamil Nadu South India. *Pakistan Journal of Nutrition* 8(1): 83 – 85.
19. Mehrorosh, H., Gavanji S., Larki, B., Mohammadi, M.D., Karbasiuin Bakhtari, A., (2014). Essential oil Composition and antimicrobial screening of some Iranian Herbal Plants and on Pectobacterium carotovoru. Global Nest J. 16: 240-50 https//doi.org/10.30955/gmj.00/205

20. Morellion, p., Que, Y.A., and Glauser, M.P., 2005. *Staphilococcus aureus* (including Staphiloccal Toxic Shock) Principles and Practices of Infectious diseases 6th ed. Vol.2. Pennyslyvania: Published by Churchil Livingstone. Pp 2333-2334.
21. Odunayo, R.A, Ibukun, E.A., Tayo, A., Toyin, A., and Tolu, O., 2007. Invitro Antimicrobial Activity of Crude Extracts from Plants Bryophyllum pinnatum and Kalanchoe crenata. *African Journal of Traditional Complementary, and Alternative Medicines.*
22. Ofokonsi, K.C, Esimone, C.O and Anele, C.K 2005. Evaluation of the In-vitro combined anti-Bacterial effects of the leaf extracts of *B. pinnatum* and *O. gratissimu.* Plant prod. Res.J.9:23-27.
23. Pretorious, J.C., Magama, S., Ziestman, P.C., 2003. Growth Inhibition of Plant Pathogenic Bacteria and Fungi by extracts from selected South African Plant Species. South African *Journal of Botany*; 20: 188-192
24. Reena and Saxena, Pra mod Patil and S.S., Khan, 2010. Screening for phytochemical analysis of *Eucalyptus globules* labill and emblica officinalis gaerrn. Namobiotechnica universal 4(5): 950 – 962.
25. Samir, F., Amal, D., Hafedh, H., Kais, M., Neji, G., and Adel H 2017. Solvent Extraction Effects on phytochemical constituents and Antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. Food Sci Technol campinas, 37(3):483-492.
26. Sandhya, B., Thomas, S., Isabel, W., Shenbagarthal, R., 2006. Ethnomedicinal Plants used by the Valaiyan community of Piranmalai hills (reserved forest) Timilnadu India: Pilot Study. *African Journal of Traditional, Complementary and Alternative Medicine*s.12;3(1): 101-14. https//doi.org/10.4314/ajtcam.v3il.31145.
27. Shaheer, T., Syed, T.A., Ghazala, H.R., Muhammad, N, Sofowore, A., 1993. Medical Plants and Traditional Medicines in Africa. Ibadan Published by Spectrum Books Ltd.
28. Yushau, M., Onuorah, F.C. and Murtala, Y. 2009. In vitro sensitivity pattern of some urinary tract isolates to *Carica papaya* extracts. *Bayero Journal of Pure and Applied Science*, 2(2): 75 – 78.
29. Tatsimo, S.J.N., Tamakou, J.D.D, Havyarima, L. 2012. Antimicrobial and Antioxidant activity of Kaempferol rhanoside derivatives from *B. pinnatum*. 5, 158 https://doi.org/10.1186/1756-05005-158
30. Williams, D., 2000. Antimicrobial resistance a global threat. Esssential Drug Monitor. 28- 29: 1.
31. World Healt Organisation (WHO), author Traditional medicine. Fact sheet number 134. Revised May, 2003.2001. available on https/www.who.int/mediacentre factsheet/134.