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Evaluation of Antibacterial and Phytochemical Properties of *Bryophyllum pinnatum* (Lam.) on Bacteria Associated with Urinary Tract Infections

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About Article

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ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections and are often caused by multidrug-resistant pathogens. With the increasing antibiotic resistance, there is a growing interest in exploring plant-based alternatives for antimicrobial therapy. *Bryophyllum pinnatum*, a medical plant known for its broad pharmacological properties, has been traditionally used for treating infections and inflammatory conditions. This study aimed to investigate the antibacterial activity and phytochemical properties of *Bryophyllum pinnatum* extracts on bacteria isolated from urinary tract infections. A total of four hundred and seventy three (473) urine samples were obtained from patients attending selected Government hospitals in Akure, Ondo-State. The isolation of bacterial organisms were performed using standard microbiological methods. Phytochemical screening was conducted using methanol, ethanol, hot water, and aqueous extracts of the plant. The results revealed the presence of saponins, tannins, phlobatannins, flavonoids, steroids, terpenoids, and cardiac glycosides in the extracts. Among the extracts, the aqueous extract exhibited the highest percentage yield at 32.17%, followed by the hot water extract at 27.23%. In contrast, methanol and ethanol extracts had lower yields of 12.36% and 12.59%, respectively. The antibacterial efficacy of the extracts was strain-dependent; specific bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*, demonstrated greater susceptibility to the extracts, resulting in larger inhibition zones. The antimicrobial activity of the extracts was evaluated against selected bacterial strains, with minimum inhibitory concentration (MIC) values ranging from 25 mg/ml to 400 mg/ml. Notably, the methanol and hot water extracts displayed significant activity against *Enterobacter aerogenes* and *E. coli* at a 25 mg/ml concentration. Minimum bactericidal concentration (MBC) values ranged from 200 mg/ml to 400 mg/ml, with the methanol extract showing the strongest bactericidal effect. Ethanol and methanol extracts produced the largest inhibition zones, measuring 17.5 mm against *Staphylococcus aureus* and 16 mm against *Klebsiella pneumoniae*, both at a concentration of 400 mg/ml. Lower concentrations (50 mg/ml and 25 mg/ml) exhibited minimal inhibitory effects. The ethanol extract demonstrated moderate antimicrobial properties, with inhibition zones varying from approximately 5 mm to 17 mm, suggesting that this solvent is effective for extracting antibacterial compounds from the plant. These findings highlight the potential of *Bryophyllum pinnatum* extracts as promising antimicrobial agents against resistant bacterial strains.

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1. INTRODUCTION

Urinary tract infections (UTIs) rank among the most prevalent infectious diseases worldwide. They occur more frequently in females than in males, with *Escherichia coli* identified as the primary causative agent in 80 - 90% of cases. Other bacterial pathogens associated with UTIs include *Staphylococcus aureus*, Enterococcus faecalis, Pseudomonas aeruginosa, *Proteus mirabilis*, and *Klebsiella pneumoniae* (Ortega-Lozano *et al.*, 2023). In recent times, the resistance of bacteria strains to antibiotics has increased due to the high cost of antibiotics and the overuse and misuse of antibiotics. This has necessitated the search for alternative medicine to cure these infections.

Bryophyllum pinnatum (Lam.), commonly referred to as the "miracle leaf" or "life plant," is a succulent belonging to the Crassulaceae family. This plant has been traditionally utilized in various cultures to treat infections, wounds, and inflammatory conditions (Akinpelu *et al.*, 2015). It is rich in phytochemicals such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, all of which exhibit antimicrobial and anti-inflammatory properties (Patil & Ghosh, 2010). These bioactive constituents contribute to its broad-spectrum antimicrobial activity against numerous bacterial and fungal pathogens.

2. LITERATURE REVIEW

Medicinal plants are economically important, accessible, and are used for treating various ailments. Researches have been conducted globally to confirm the efficacy of medicinal plants, and part of this study has provided light on the synthesis of plant-based chemicals with potential therapeutic uses (Dhama *et al.*, 2014). *Bryophyllum pinnatum* is commonly referred in English as "never die" plant, "life miracle," or "resurrection." In Nigeria, it is locally known as Karan (in Hausa), Ugwoba (in Igbo), and Abamoda (in Yoruba) (Bassey *et al.*, 2021).

The plant has numerous macro and microelements, vitamins, calcium, phosphorus, ascorbic acid, and inulin (Okwu & Josiah, 2006). This plant is employed by numerous traditional health practitioners across various global regions as a therapeutic agent for a plethora of conditions, including hypertension, dermatological disorders, neoplasms, pyrexia, diabetes mellitus, hepatitis, abscess formation, wound repair, and otitis media (Rajsekhar *et al.*, 2016). Many investigations have been conducted to elucidate the phytochemical composition of *B. pinnatum*, uncovering a rich array of bioactive constituents. Among these are flavonoids, which are recognized for their antioxidative and antimicrobial functions (Fernandes *et al.*, 2019). In particular, extracts derived from *B. pinnatum* have been documented to encompass quercetin and kaempferol, flavonoids with well-established biological activities (Ibitoye *et al.*, 2018).

B. pinnatum has also been shown to include lipids, organic acids, phenols, triterpenes, alkaloids, glycosides, and bufadienolides (Thorat *et al.*, 2018). However, because bufadienolides may be unsafe, their presence should be treated with caution (Frer *et al.*, 2016). Several factors, including the plant component employed, geographical location, and the extraction techniques, might affect the precise makeup of these phytochemicals (Latif *et al.*, 2020).

The bioactive constituents present in the leaf extract of *B. pinnatum* exhibit significant medicinal properties against a variety of infections, including otitis media (Akinpelu, 2000). Empirical investigations have demonstrated that *B. pinnatum* possesses notable antibacterial efficacy against a range of pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and Pseudomonas aeruginosa. Various solvents, including aqueous, ethanol, methanol, and n-hexane, have been employed for the extraction process, and the resultant extracts have revealed differing levels of antibacterial activity against the specified pathogens (Mudi *et al.*, 2008).

The antibacterial efficacy of *B. pinnatum* extracts has been systematically assessed against multiple bacterial strains, particularly those frequently implicated in urinary tract infections (UTIs). Numerous investigations have substantiated the presence of significant inhibitory effects against *E. coli*, *S. aureus*, *S. saprophyticus*, and *P. aeruginosa* (Mabeku *et al.*, 2016). The proposed mechanism for this antimicrobial effect can include both antioxidant properties that provide defense against reactive oxygen species and direct bactericidal activities (Mabeku *et al.*, 2016). Further studies have also reported that *B. pinnatum* exhibits antibacterial properties against *S. aureus* and *E. coli*, with varying levels of effectiveness depending on the extraction process and extract concentration (Romulo *et al.*, 2018; Bereksi *et al.*, 2018). This study explores the antimicrobial activity and phytochemical properties of *Bryophyllum pinnatum* on bacteria isolated from urinary tract infections.

3. METHODOLOGY

3.1. Collection of plant Leaves

Fresh leaves of *Bryophyllum pinnatum* were collected from the Federal University of Technology Akure, located in Ondo State, Nigeria in the month of April, 2023. The botanical specimen was identified and authenticated by a botanist at the Federal Institute of Forestry in Ibadan, Oyo State, with the assigned voucher number FHI 114111.

3.2. Extraction Procedure

After being thoroughly washed with distilled water, the plant leaves were allowed to be air-dried at a temperature of 30°C for about two weeks, and crushed into powder form using a mechanical grinder. Methanol, Ethanol, water and hot water extraction were used for the extraction. A quantity of 100 grams of the dried powder was measured individually, immersed in 1000ml of methanol, ethanol, water and hot water for 72 hours, and subjected to constant agitation to facilitate the extraction of the powdered sample. Filter paper (Whatman No. 1) was employed to filter the extract mixtures, and a vacuum rotary evaporator was utilized for concentration purposes.

3.3. The percentage recovery yield is typically calculated using the following formula:

Percentage Recovery (%) = (Weight after Extraction / Weight before Extraction) × 100

3.4. Ethical Statement

Ethical clearance was obtained from the Ondo State Health Research Ethics Committee (OSHREC), Ondo State Ministry of Health, with the protocol number OSHREC 15/04/2023. Before

the collection of the samples, the consent forms were filled, and the participants were enrolled for the study.

3.5. Collection and culturing of Urine samples

A total of four hundred and seventy-three (473) urine samples were collected from selected healthcare facilities in Akure, specifically the University of Medical Science Teaching Hospital (UNIMED), along with Basic Health Centers located in Isolo and Orita-obele within Akure, Ondo State. The urine samples were transported aseptically to the Microbiology laboratory Federal University of Technology were it was cultured, isolated, and identified. The bacterial species identified included *Staphylococcus aureus*, *Escherichia coli*, Staphylococcus saprophyticus, *Klebsiella pneumoniae*, Enterobacter aerogenes, Pseudomonas aeruginosa, Micrococcus luteus, *Proteus mirabilis*, Enterococcus faecalis, and Proteus vulgaris.

3.6. Antibacterial activity of the extracts against bacteria isolates

The selected multidrug-resistant organisms, which had previously been subjected to the standard antibiotics, were subsequently evaluated against the extract derived from medicinal plants. The extracts were dissolved in 1% DMSO and subjected to sterilization utilizing membrane filters with a pore size of 0.45 µm. The filtrate was preserved in a refrigerator at 4°C for subsequent use. The antimicrobial bioassay was done using the agar well diffusion methods. All bacterial isolates were inoculated at a temperature of 37°C for 18 hours, with their turbidity being adjusted to the equivalent of the 0.5 McFarland standard. Suspensions of the bacterial strains were introduced onto Mueller Hinton agar plates utilizing a sterile swab. The swab was uniformly applied over the medium's surface to enhance confluent growth. A core borer with a diameter of 6 mm was employed to create uniform wells within each of the Mueller Hinton plates. Approximately 0.5 ml of the various diluted extracts were dispensed into the wells. An equal amount of DMSO was utilized as a negative control, and all plates were incubated at 37°C for 24 hours to assess the zone of inhibition elicited by the extract.

3.7. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extracts was ascertained through a method of serial doubling dilution. A volume of 5 ml of nutrient broth was measured into sterile tubes, to which one milliliter of various concentrations of the extract was subsequently incorporated and then inoculated with a test organism derived from standardized 0.5 McFarland suspensions of the bacteria; this mixture was then incubated at 37°C for a duration of one night. The tube exhibiting the lowest concentration of the extract that displayed no growth was identified as the "MIC."

3.8. Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) of the extracts against bacteria was evaluated by streaking the surface of Müller Hinton agar plates with non-turbid tubes obtained from the MIC assessment. Following an overnight incubation period, the plates were examined for any signs of bacterial growth. The lowest concentration that demonstrated the absence of growth was considered as the MBC.

3.9. Phytochemical screening

The plant extracts were screened quantitatively according to the method described by Harborne 1998; Evans, 2009. The phytochemicals analyzed were steroids, alkaloids, flavonoids, saponins, tannins, glycosides, anthraquinone, phenols, and cardiac glycosides.

The method described by (Brunner, 1994; Sofowora, 1993) were used to determine the quantitative phytochemical analysis of the plant extracts

3.10. Statistical analysis

Results were expressed as the mean ± standard deviation (SD). Data analysis was done using one-way analysis of variance (ANOVA), and the means were compared using Duncan's New Multiple range test at $P \leq 0.05$ level of significance (SPSS version 17).

4. RESULTS AND DISCUSSION

4.1 Percentage Recovery of Plant Extracts

The percentage recovery of Bryophylum pinnatum extracts is shown in (Table 1). Aqueous extract (32.17%) had the highest yield percentage among the extracts, followed by Hot water extract (27.23%), while the lowest yield was seen in methanol (12.36%) and ethanol (12.59%), respectively.

Table 1. Percentage Recovery of Plant Extracts

Plant extracts	Weight before extraction (g)	Weight after extraction (g)	Percentage recovery (%)
BPM	100	12.36	12.36
BPE	100	12.59	12.59
BPA	100	32.17	32.17
BPH	100	27.23	27.23

Keys: BPM= Bryophyllum pinnatum; BPE= Bryophyllum pinnatum ethanol; BPA= Bryophyllum pinnatum aqueous; BPH= Bryophyllum pinnatum

4.5. Quantitative Analysis of Phytochemical Content of *Bryophyllum Pinnatum*

The results of qualitative phytochemical screening of methanol, ethanol, hot and aqueous extracts of *Bryophyllum pinnatum* as represented in (Table 2) revealed the presence of saponin, tannin, phlobatannin, flavonoid, steroid and terpenoid, alkaloid and cardiac glycosides. However, alkaloids were absent in the methanol extract, and anthraquinones were absent in all the extracts.

Table 2. Quantitative Analysis of the Phytochemical Content of *Bryophyllum pinnatum*

Phytochemicals(mg/ml)	METHANOL	ETHANOL	HOT	AQUEOUS
Saponin	+	+	+	+
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+
Steroid	+	+	-	-
Terpenoid	+	+	+	+
Alkaloid	-	+	+	+
Anthraquinone	-	-	-	-
Cardiac glycosides				
Legal test	+	+	+	+
Keller kiliani test	+	+	+	+
Salkwoski test	+	+	+	+
Lieberman test	+	+	-	-

+=Positve - =Negative

4.3. Quantitative Constituents of Phytochemical Content *Bryophyllum pinnatum*

The results of the quantitative phytochemical screening of methanol, ethanol, hot and aqueous extracts of *bryophyllum pinnatum* in (Table 3) shows that the concentration of saponin ranged from (12.90 ± 0.6^{a}) to (21.81 ± 0.26^{b}), with ethanol having the highest concentration of (21.81 ± 0.26^{b}). Steroids were absent in both aqueous and hot extracts. In addition, the absence of alkaloids was recorded in the methanol extract of the plant.

Table 3. Quantitative Constituents of Phytochemical Content *Bryophyllum pinnatum*

	SAPONIN	TANIN	FLAVONOID	TERPENOID	STEROID	ALKALOID	GLYCOSIDE
AQUEOUS	12.90 ± 0.6^{a}	0.53 ± 0.01^{a}	0.25 ± 0.01^{c}	4.30 ± 0.03^{a}	0.00 ± 0.00^{a}	0.62 ± 0.03^{d}	2.73 ± 0.04^{b}
HOT	12.18 ± 0.24^{a}	0.59 ± 0.01^{b}	0.24 ± 0.01^{bc}	4.62 ± 0.04^{b}	0.00 ± 0.00^{a}	0.38 ± 0.03^{c}	2.63 ± 0.04^{b}
METHANOL	20.90 ± 0.26^{b}	0.67 ± 0.01^{c}	0.20 ± 0.01^{a}	4.81 ± 0.04^{c}	1.09 ± 0.19^{b}	0.00 ± 0.00^{a}	2.40 ± 0.04^{a}
ETHANOL	21.81 ± 0.26^{b}	0.61 ± 0.01^{b}	0.22 ± 0.01^{ab}	4.78 ± 0.03^{c}	1.30 ± 0.24^{b}	0.25 ± 0.03^{b}	2.34 ± 0.04^{a}

Values represent means ± Standard deviation of duplicates reading. Superscript of the same letter in the row are not significantly different at ($P\leq0.05$) according to new Duncan's multiple range test.

4.4. Minimum Inhibitory Concentration of *Bryophyllum Pinnatum* (MIC mg/ml)

The minimum inhibitory concentration value of *Bryophyllum pinnatum* extracts against the selected bacterial strains are presented in (Table 4). The MIC for aqueous extract ranged from 100mg/ml to 400mg/ml, hot water 25mg/ml to 400mg/ml, ethanol extract 100 mg/ml to 400mg/ml, and methanol extract 25mg/ml to 400mg/ml respectively. Hot water extract and methanol extract were active against Enterobacter aerogenes, *E. coli* 2 and methanol extract against *E. coli* 2 at the range 25mg/ml. Some of the bacteria isolates (MIC) were not detected (ND) at different extracts shown below.

Table 4. Minimum Inhibitory Concentration of *Bryophyllum Pinnatum* (MIC mg/ml)

Isolates	Aqueous Extract	Hot Water Extract	Ethanol Extract	Methanol Extract
Escherichia coli1	ND	50	400	200
Enterobacter aerogenes	200	25	ND	50
*Staphylococcus aureus*1	400	50	400	50
Enterococcus faecalis	400	50	200	50

Isolates	Aqueous Extract	Hot Water Extract	Ethanol Extract	Methanol Extract
Escherichia coli 2	100	25	200	25
Micrococcus luteus	200	ND	ND	400
Klebsiella pneumonia	ND	200	100	200
Proteus mirabilis 2	400	200	400	200
Pseudomonas aeruginosa	ND	50	ND	50
Staphylococcus aureus	ND	400	200	ND
Proteus vulgaris	400	200	400	100
Proteus mirabilis 1	ND	400	200	ND
S. saprophyticus	400	400	100	100
Enterococcus faecalis2	200	200	100	400

4.5. Minimum Bacteriocidal Concentration of *Bryophyllum pinnatum* (MBC mg/ml)

The minimum bactericidal concentration revealed that all the extracts (aqueous extract, methanol, ethanol and hot water) ranged from 50mg/ml to 400 mg/ml. MBC values for methanol and hot water extract were lower than other extracts, indicating a stronger bactericidal effect.

Table 5. Minimum Bacteriocidal Concentration of *Bryophyllum pinnatum* (MBC Mg/ml)

Isolates	Aqueous extract	Hot water extract	Ethanol Extract	Methanol Extract
Escherichia coli 1	ND	400	ND	400
Enterobacter aerogenes	ND	200	ND	200
*Staphylococcus aureus*1	ND	200	ND	100
Enterococcus faecalis 1	400	ND	ND	400
*Escherichia coli*2	400	50	400	50
Micrococcus luteus	ND	ND	ND	ND
Klebsiella pneumonia	ND	ND	400	400
Enterococcus faecalis 2	400	ND	ND	ND
*Proteus mirabilis*2	400	ND	400	ND
Pseudomonas aeruginosa	ND	400	ND	400
Staphylococcus aureus	ND	400	400	400
Proteus vulgaris	400	ND	400	200
Proteus mirabilis 1	ND	400	200	ND
S. saprophyticus	400	ND	200	ND
Escherichia coli 1	ND	400	400	ND
Enterobacter aerogenes	ND	200	ND	200

ND- Not detectable

4.6. Antimicrobial activity of *Bryophyllum pinnatum* hot water extraction

The *Bryophyllum pinnatum* extract of hot water demonstrated strong inhibitory effects against E.coli 2 at 400mg/ml and also against E. aerogenes across all the concentrations. Weak inhibitory was recorded at 50mg/ml and 25mg/ml.

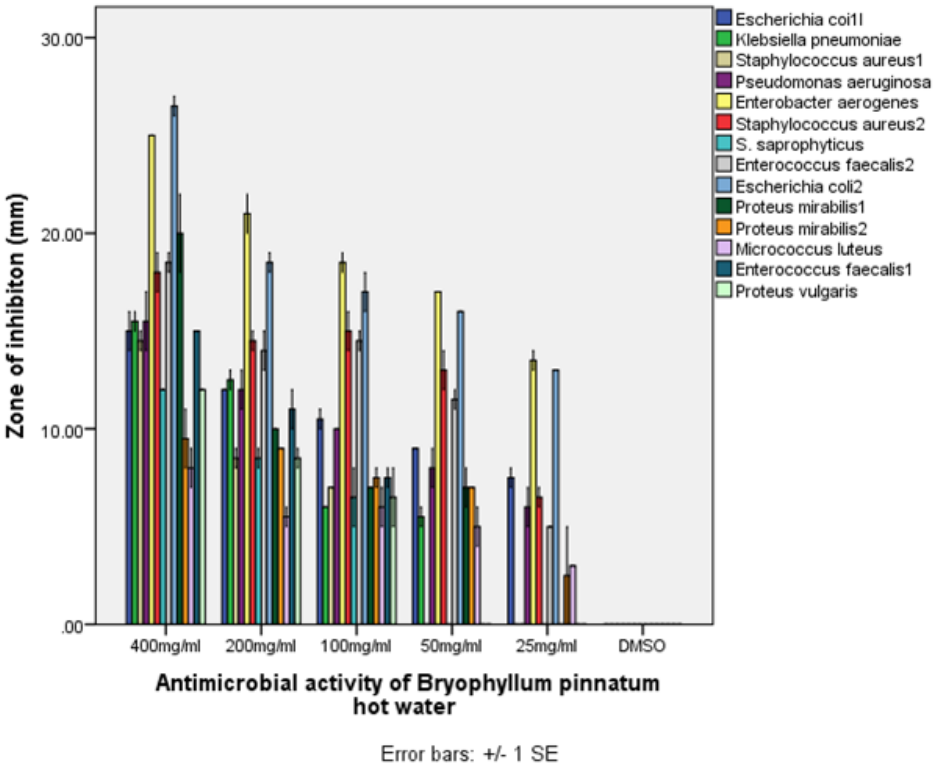

Figure 1. Antimicrobial activity of *Bryophyllum pinnatum* hot water extract

4.7. Antimicrobial activity of *Bryophyllum pinnatum* aqueous water extract

The aqueous extract showed the highest zone of inhibition at concentrations of 400 mg/mL and 200 mg/ml. Zone of inhibition were not detected at concentrations of 50 mg/ml, 25 mg/ml. Staphylococcus saprophyticus and *Escherichia coli*, exhibited relatively higher susceptibility to the extract, as indicated by a large inhibition zone. In contrast, at 100mg/ml Klebsiella *Proteus mirabilis* 1 exhibited a reduced susceptibility.

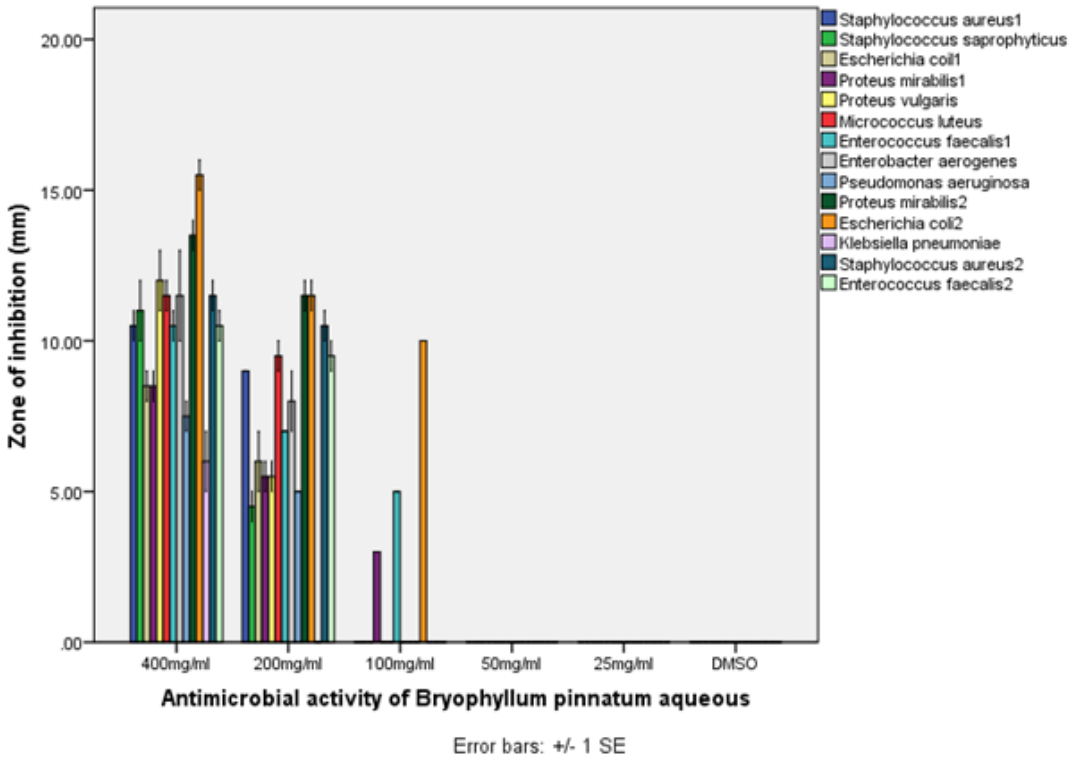

Figure 2. Zone of inhibition of *Bryophyllum pinnatum* aqueous extract against selected bacteria

4.8. Antimicrobial activity of *Bryophyllum pinnatum* ethanol extract

The ethanol extract of *Bryophyllum pinnatum* demonstrates antimicrobial activity against certain bacterial strains. The most significant zone of inhibition was observed with *Staphylococcus aureus*, measuring 17.5 mm at a concentration of 400 mg/ml. This was followed by *Klebsiella pneumoniae*, which exhibited a zone of inhibition of 16 mm at the same concentration. At a concentration of 100 mg/ml, the tested bacteria showed a smaller zone of inhibition, while no inhibition was recorded at 25 mg/ml.

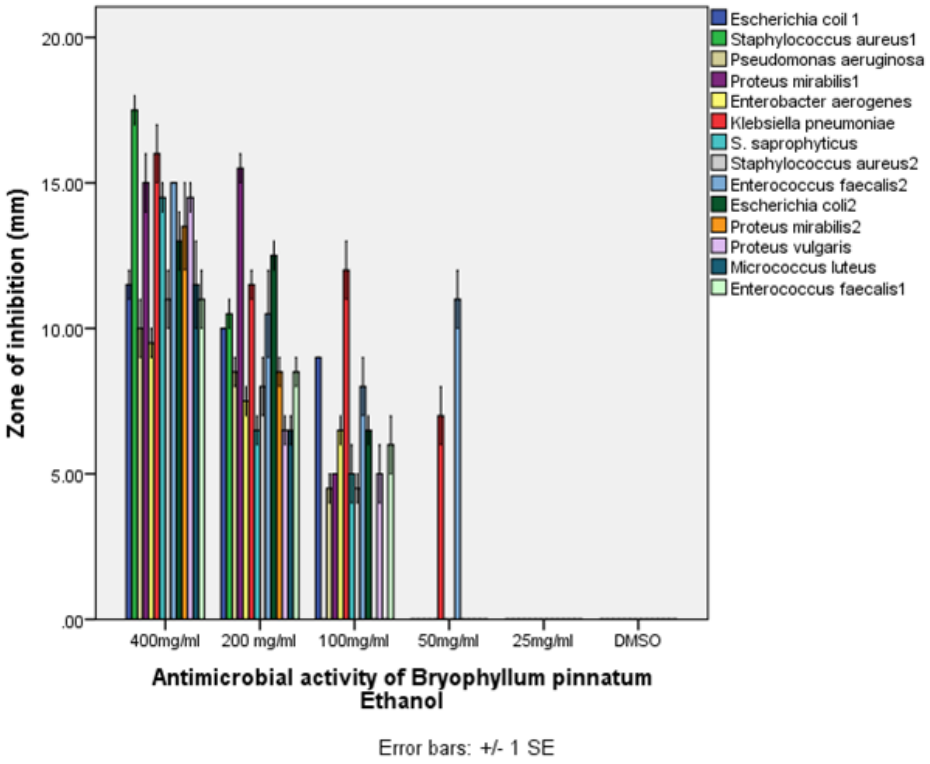

Figure 3. Zone of inhibition of *Bryophyllum pinnatum* ethanol extract against selected bacteria

4.9. Antimicrobial activity of *Bryophyllum pinnatum* ethanol extract

Higher concentrations, specifically 400 mg/ml and 200 mg/ml, showed significantly larger zones of inhibition among the bacterial isolates. In contrast, lower concentrations (50 mg/ml and 25 mg/ml) displayed minimal inhibition. Among the bacterial strains tested, *Escherichia coli*, *Staphylococcus aureus*, and Pseudomonas aeruginosa exhibited relatively higher susceptibility to the extract, resulting in larger inhibition zones. Conversely, other strains such as *Proteus mirabilis* and Enterococcus faecalis, showed only moderate inhibition. *Proteus mirabilis* and Enterococcus faecalis displayed moderate inhibition.

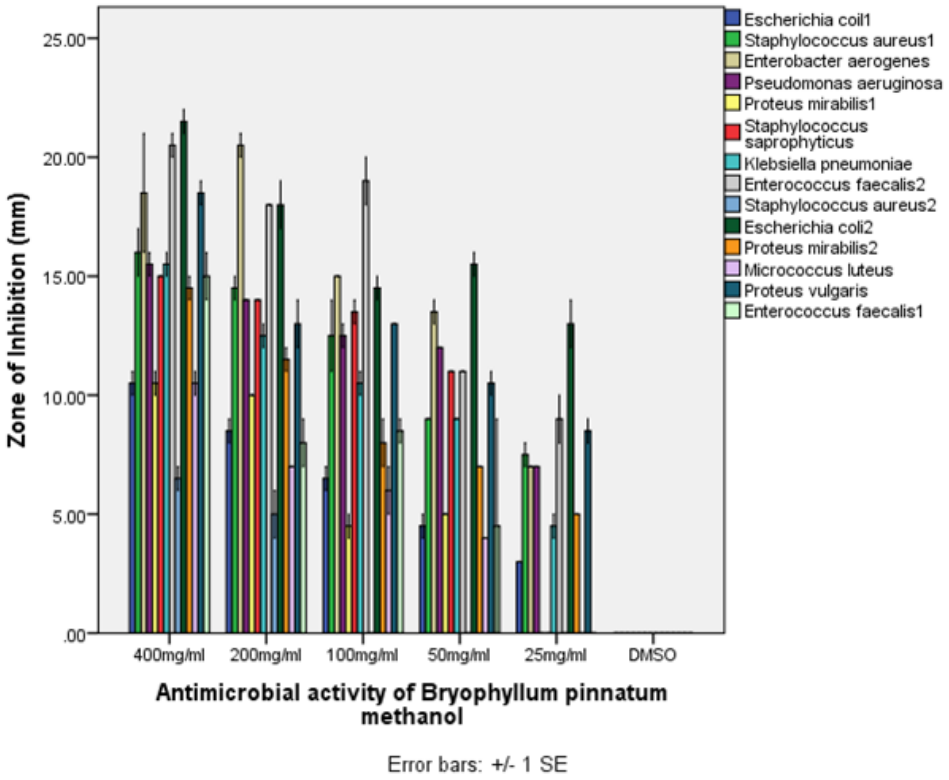

Figure 4. Antimicrobial activity of Bryophyllum pinnatum methanol extract

4.10. Discussion

The increase in the resistance of bacteria pathogens has become a global health threat. The phytochemical screening of *Bryophyllum pinnatum* extracts (methanol, ethanol, hot water, and aqueous) showed the presence of saponin, tannin, flavonoid, terpenoid, and alkaloid. This is similar to the findings reported by Izundu *et al.* (2021).

This study evaluates the percentage recovery of four plant extracts: BPM (*Bryophyllum pinnatum* methanol), BPE (*Bryophyllum pinnatum* ethanol), BPA (Bryophyllium pinnatum aqueous), and BPH (*Bryophyllum pinnatum* hot). According to the findings, BPA had the highest extraction yield (32.17%), followed by BPH (27.23%), while BPM (12.36%) and BPE (12.59%) had much lower recoveries. The variability in extraction yield may be due to variations in the phytochemical composition, the solubility of the compounds in the extraction solvent, and the effectiveness of the extraction technique employed.

The high recovery percentages for BPH and BPA suggest either a more potent solvent-plant interaction or a higher concentration of extractable compounds. Research has indicated that when employing the most effective plants, those that are strong in secondary metabolites, such as flavonoids, tannins, and alkaloids, generally have better extraction yields (Sasidharan *et al.*, 2011).

Numerous investigations have demonstrated the antibacterial properties of *B. pinnatum*, with various solvent extracts exhibiting different levels of efficacy. In this study, methanol and hot water extract has shown antibacterial activity against the selected bacteria more than ethanol and aqueous extracts. The hot water (25–50 mg/ml) and methanol (25–50 mg/ml) extracts exhibited the lowest minimum inhibitory concentration (MIC) values, indicating the highest antibacterial effectiveness, particularly against *E. coli* 2, E. aerogenes, S. aureus 1, and E. faecalis.

Since many bioactive compounds are poorly soluble in water, aqueous extracts often prove to be less effective (Cowan, 1999). In the assessment of antimicrobial efficacy, the aqueous extract demonstrated relatively modest activity, with the majority of inhibition zones measuring less than 15 mm. Notably, the antimicrobial activity was more pronounced at higher concentrations of 400 mg/ml and 200 mg/ml; however, it significantly diminished at concentrations of 100 mg/ml and lower. While some bacterial strains, including Micrococcus luteus and *Proteus mirabilis*, were susceptible to the extracts, the resulting inhibition zones were notably smaller when compared to those obtained from ethanol and hot water extracts. Ogunbanwo *et al.* (2020) found that aqueous extracts displayed minimal antimicrobial activity, which aligns with the reduced inhibition zones noted in the figure depicting the aqueous extract results. Aqueous extracts exhibit limited antimicrobial activity, likely attributable to their inability to solubilize certain hydrophobic bioactive compounds (Eloff, 1998).

The ethanol extract displayed moderate antimicrobial properties, with inhibition zones ranging from approximately 5 mm to 17 mm. Higher concentrations (400 mg/ml and 200 mg/ml) demonstrated increased inhibition, while lower concentrations (50 mg/ml and 25 mg/ml) exhibited minimal effects. The antimicrobial efficacy was found to be strain-dependent, as certain bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*, showed greater susceptibility. Akinsulire (2007) indicated that the alcoholic extracts of *B. pinnatum* demonstrated antimicrobial activity

against several Gram (+ve) and (-ve) bacterial strains.

Also, the study demonstrated that the extract obtained through hot water extraction exhibited the most significant antimicrobial activity, with inhibition zones measuring between 25 to 30 mm. This suggests that hot water extraction is effective for yielding bioactive compounds, as its antimicrobial activity surpassed that of ethanol. Notably, certain bacterial strains, such as Pseudomonas aeruginosa and *Klebsiella pneumoniae*, showed high susceptibility to this extract. Furthermore, research on medicinal plants has indicated that hot water extracts can sometimes surpass those obtained through organic solvents. For instance, Ezekiel *et al.* (2021) demonstrated that boiling can enhance the bioactive compounds present in these extracts.

The methanol extract also displayed considerable antimicrobial efficacy. Its inhibition zones ranged from 10 mm to 25 mm, indicating that methanol serves as an effective solvent for extracting antimicrobial compounds from *Bryophyllum pinnatum*. Most bacterial strains exhibited susceptibility to this extract, particularly at higher concentrations.

Numerous studies have validated the antimicrobial properties of *Bryophyllum pinnatum*. Research conducted by Akinpelu *et al.* (2017) demonstrated that methanol extracts from the plant exhibited significant antibacterial effects, corroborating the findings presented in this study. Karabi and Sankar (2015) demonstrated that methanolic extracts exhibit significant antibacterial properties against highly resistant urinary tract infection (UTI) isolates and Pseudomonas aeruginosa. Similarly, Praveen (2012) found that methanol extracts of *B. pinnatum* displayed marked antibacterial activity against multidrug-resistant *E. coli*, with inhibition zones ranging from 8 mm to 22 mm. Akinpelu (2000) reported that *B. pinnatum* extracts possess antimicrobial properties, which may be attributed to bioactive compounds in these plant extracts.

Additionally, Dholaria and Desai (2014) indicated that while methanol extracts showed the highest effectiveness against various selected isolates, ethanol extracts demonstrated the greatest activity against Pseudomonas aeruginosa.

5. CONCLUSIONS

Bryophyllum pinnatum exhibits antibacterial and phytochemical attributes that may be beneficial in treating urinary tract infections. The plant contains a range of bioactive compounds, including flavonoids and phenols, which contribute to its antimicrobial effectiveness. This plant exerts its effects through various mechanisms, including disruption of bacterial membranes, interference with enzymatic pathways, and the induction of oxidative stress, thereby demonstrating a multifaceted approach against bacteria pathogen. In vitro investigations have indicated its efficacy against prevalent uropathogens. However, further clinical trials are essential to establish its efficacy and safety in humans.

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