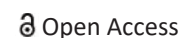


RESEARCH ARTICLE



Antimicrobial activity of the ethanol extract of *Bryophyllum pinnatum* leaves on multi-drug resistant bacterial strains

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ABSTRACT

Aim: The rise in drug resistance has underscored the necessity of exploring alternatives to conventional medications. Herbal remedies, which have gained societal acceptance, represent a significant avenue for such exploration. This study focuses on assessing the antibacterial activity of the ethanolic leaf extract of *Bryophyllum pinnatum* against some multi-drug resistant (MDR) bacteria.

Methods: The ethanolic extract was prepared from fresh *B. pinnatum* leaves using standard laboratory procedures. Antibacterial activity was assessed through the agar well diffusion method against a selection of MDR bacteria, namely *Escherichia coli*, *Proteus* spp, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method.

Results: The ethanolic extract exhibited notable antibacterial activity, particularly against *Proteus* spp and *S. typhi*. At concentrations of 200 mg/ml, inhibition zones of 18 and 24 mm were observed, while at 100 mg/ml, zones of 11 and 20 mm were recorded, respectively. The MIC test revealed that the extract was active against *S. typhi* and *Proteus* sp at 200 mg/ml. The MBC test revealed that at 200 mg/ml, the extract was active against *Proteus* sp.

Conclusion: *B. pinnatum* ethanolic leaf extract showed antibacterial activities against MDR *E. coli*, *P. aeruginosa*, and *S. aureus*, thus, making it a potential drug development candidate for the treatment of ailments caused by these pathogens. Further research is recommended to delve into the specific antibacterial components and mechanisms of action for a more comprehensive understanding.

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Introduction

Plants, as repositories of essential nutrients and a plethora of bioactive compounds, play a pivotal role in conferring benefits to both human health and the well-being of other plant species [1,2]. Notably, within the vast array of botanical resources, certain plant-derived materials exhibit remarkable antibacterial activity, offering a beacon of hope in the context of the prevailing antibiotic resistance crisis [3]. This optimism stems from the fact that many of these plant materials harbor a myriad of chemical

sites, rendering it challenging for microbial entities to evolve resistance [3]. Among such plants, *Bryophyllum pinnatum* emerges as a particularly intriguing candidate, showcasing potent antibacterial activity and positioning itself as a potential alternative to conventional antibiotics that may have been rendered inactive due to resistance mechanisms [4–6].

Bryophyllum pinnatum, commonly known as the “miracle leaf” or “air plant,” stands out as a resilient and versatile succulent perennial, belonging to the *Crassulaceae* family [7]. Native to Madagascar, this

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resilient and versatile plant has spread to various tropical and subtropical regions worldwide [8]. It is characterized by distinctive fleshy, serrated leaves that are capable of producing new plants through a unique form of asexual reproduction [7].

The historical significance of *B. pinnatum* extends across diverse cultures, where it has been harnessed for an array of medicinal purposes. Its applications span traditional uses in alleviating respiratory conditions, promoting wound healing, and acting as an effective anti-inflammatory and analgesic agent [7,9]. Beyond its medicinal prowess, the plant assumes cultural and ritualistic importance, symbolizing resilience and finding utility in ceremonial and ornamental contexts [8,9]. This multifaceted role underscores the profound connection of the plant with human societies throughout history.

Scientific interest in *B. pinnatum* has spurred extensive research into its chemical composition and potential therapeutic applications in the realm of modern medicine [9–11]. The pharmacological properties and bioactive constituents of the plant have been subjects of rigorous investigation, seeking to unravel the intricate mechanisms that underlie its diverse medicinal attributes [9,11]. While its ethanolic leaf extract has demonstrated promising antibacterial properties, ongoing research endeavors seek to deepen our understanding of its effects on multi-drug resistant (MDR) bacteria. Multi-drug resistance occurs when an organism exhibits resistance to multiple antimicrobial agents, and this poses a significant global health concern [12].

In the present study, we assessed the antibacterial activity of the ethanolic leaf extract of *B. pinnatum* against some MDR bacteria, including *Escherichia coli*, *Proteus* spp, *Pseudomonas aeruginosa*, *S. typhi*, and *Staphylococcus aureus*. This research aims to contribute significantly to the evolving discourse on antibiotic resistance, unraveling the potential of *B. pinnatum* as a valuable agent in the ongoing battle against MDR bacterial infections. Through this exploration, we aspire to lay the groundwork for future therapeutic strategies that harness the untapped potential of herbal medicine.

Materials and Methods

Plant material collection and extraction

This study utilized fresh *B. pinnatum* leaves procured from Samaru market, Zaria, Kaduna State. The identification was carried out at the Department of Horticulture, Kaduna State University, Nigeria, and

assigned the voucher number KASU/BSH/1620. Subsequently, the leaves underwent air-drying at room temperature, followed by pulverization into a coarse powder using a mortar and pestle for extraction purposes.

The extraction process involved cold maceration, where 200 g of the pulverized leaves were macerated in 1 l of ethanol. The resulting mixture underwent filtration using Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK) and was concentrated to dryness in a water bath. Following this, the extract was weighed and preserved in a refrigerator at 4°C until it was utilized for subsequent analyses.

Analyses for proximate, anti-nutrient, and phytochemical compositions

The study analyzed the proximate compositions (moisture content, ash, crude fiber, protein, and fat) of *B. pinnatum* leaves using the standard AOAC (2005) methods [13]. For the determination of anti-nutrients (phytate, tannin, and oxalate), titrimetric methods as outlined by Abdi *et al.* were employed [14]. In addition, the phytochemical compositions of the extract were determined using methods described by Trease and Evans [15].

Extract preparation for antibacterial assay

The extract was dissolved in 10 ml of dimethyl sulfoxide to create a concentration of 200 mg/ml, using 2 g of the extract. Subsequently, this concentration was further prepared by ten-fold serial dilution to obtain concentrations of 100, 50, and 25 mg/ml. These varying concentrations were then utilized for the antibacterial assay.

Bacterial organisms used for the assay

Selected MDR bacteria were used and these included *E. coli*, *Proteus* spp, *Pseudomonas aeruginosa*, *S. typhi*, and *Staphylococcus aureus*.

Antibacterial assay

Nutrient Agar media was prepared following the manufacturer's instructions. Subsequently, the agar was uniformly streaked with a 1 McFarland standardized suspension of the test organisms, appropriately labeled. Holes were then bored into the culture media using a sterile cup borer (6 mm). To prevent the spread of the extracts, the bottom of each well was sealed with a drop of molten agar.

Next, 1 ml of the prepared extracts was added to each well, and the cultures were left undisturbed

for 30 minutes to allow for solidification before transferring them to the incubator. Following a 24-hours incubation period at 37°C, the cultures were examined for final readings. Control plates were also prepared for the test organisms, omitting the addition of extracts. In addition, a positive control was established using streaked media and Ofloxacin. The inhibition zones were measured to the nearest millimeter, as outlined by Cheesbrough [16].

Determination of minimum inhibitory concentration (MIC)

The MIC of the extract was determined using the tube dilution method with nutrient broth as the diluent. Each tube, comprising 5 ml of broth and 2 ml of the extract, was inoculated with approximately 1 ml of each organism. The inoculated tubes were then incubated at 37°C for 24 hours. Subsequently, the tubes were examined for the presence or absence of turbidity (indicating growth). The lowest concentration in the series without any visible signs of growth was identified as the MIC [17].

Determination of minimum bactericidal concentration (MBC)

The MIC result was employed to ascertain the MBC. Samples obtained from the tubes without observable growth in the MIC assay were sub-cultured onto freshly prepared nutrient agar plates, which were subsequently incubated at 37°C for 24 hours. This step aimed to discern whether the antimicrobial effects of the extracts were bactericidal or bacteriostatic [16].

Data analysis

Data were presented using tables.

Results

Proximate composition, yield of extract, and phytochemical constituents

Out of the 200 g of *B. pinnatum* leaves, 8.50 g was obtained from the extract and the percentage yield was 4.25% (Table 1). The proximate compositions of *C. olitorius* leaves were carbohydrate (61.98%), crude fiber (12.8%), crude protein (5.2%), ash content (10.0%), crude fat (2%), and moisture content (8%). The anti-nutrients present were tannins and phytate, while oxalate was absent (Table 1). The phytochemical constituents were alkaloids, tannins, flavonoids, saponins, quinones, steroids, and terpenoids (Table 1).

Antibacterial assay

The extract showed no activity against *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table 2). There were antibacterial activities against *S. typhi* at 200 mg/ml (20 mm) and 100 mg/ml (18 mm); and *Proteus* sp at 200 mg/ml (18 mm) and 100 mg/ml (11 mm) (Table 2).

The MIC test showed that the extract was active against *S. typhi* and *Proteus* sp at 200 mg/ml, with no activity against the other isolates (Table 3).

The MBC showed that the concentration of the extract at 200 mg/ml was active against *Proteus* sp (Table 4).

Discussion

The proximate, anti-nutrient, and phytochemical compositions of the ethanolic extract from *B. pinnatum* leaves in this study showed variations when compared to other studies [18–20]. These differences can be attributed to varying geographical locations, soil types, climatic conditions, collection

Table 1. Yield and proximate, anti-nutrient, and phytochemical compositions of the ethanolic extract of *B. pinnatum* leaves collected in Zaria, Kaduna State, Nigeria.

Yield	Proximate (%)	Anti-nutrients	Phytochemicals
Weight (8.50 g)	Moisture content (8.0)	Tannins (+)	Alkaloids (+)
Percentage (4.25%)	Ash content (10.0)	Phytate (+)	Tannins (+)
	Crude protein (5.22)	Oxalate (-)	Flavonoids (+)
	Crude fat (2.0)		Saponins (+)
	Crude fibre (12.8)		Quinones (+)
	Carbohydrate (61.98)		Steroid (+)
			Terpenoids (+)

+ = present; - = absent

Table 2. Antibacteria activity (zone of inhibition) of various concentrations of *B. pinnatum* ethanolic leaf extract against some MDR bacteria in Zaria, Kaduna State, Nigeria.

	Extract concentration				
	Control (Ofloxacin)	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
	Zone of inhibition (mm)				
<i>E. coli</i>	33.00	0	0	0	0
<i>Proteus</i> sp	35.00	18.00	11.00	0	0
<i>Streptococcus pneumonia</i>	37.00	0	0	0	0
<i>S. typhi</i>	24.00	20.00	18.00	0	0
<i>Pseudomonads aeruginosa</i>	35.00	0	0	0	0

Table 3. MIC of *B. pinnatum* ethanolic leaf extract against some MDR bacteria in Zaria, Kaduna State, Nigeria.

	Extract concentration			
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
<i>E. coli</i>	ND	ND	ND	ND
<i>Proteus</i> sp	-	+	ND	ND
<i>Streptococcus pneumonia</i>	ND	ND	ND	ND
<i>S. typhi</i>	-	+	ND	ND
<i>Pseudomonads aeruginosa</i>	ND	ND	ND	ND

- = No growth; + = Growth; ND = Not determined

Table 4. MBC of *B. pinnatum* ethanolic leaf extract against some MDR bacteria in Zaria, Kaduna State, Nigeria.

	Extract concentration			
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
<i>E. coli</i>	ND	ND	ND	ND
<i>Proteus</i> sp	-	+	ND	ND
<i>Streptococcus pneumonia</i>	ND	ND	ND	ND
<i>S. typhi</i>	+	+	ND	ND
<i>Pseudomonads aeruginosa</i>	ND	ND	ND	ND

- = No growth; + = Growth; ND = Not determined

seasons, extraction methods, and the types of tests conducted [21,22].

In this study, the ethanolic extracts of *B. pinnatum* showed no antimicrobial activities against MDR *E. coli*, *P. aeruginosa*, and *S. aureus*. However, variable degrees of activity were observed against *S. typhi* and *Proteus* sp at concentrations of 200 and 100 mg/ml, as evidenced by clear zones of inhibition after incubation. Similarly, the ethanolic extracts of dried *B. pinnatum* leaves were reported to show no antibacterial activity against clinical isolates of *E. coli* and *Klebsiella pneumoniae* from patients with urinary tract infections [23]. In contrast, a previous report on the methanol extract of *B. pinnatum* leaves indicated antimicrobial activity against *E.*

coli and *S. aureus* [6]. In a study by Akinpelu [24], *B. pinnatum* leaf 60% methanolic extract was found to inhibit the growth of five out of eight bacteria used, at a concentration of 25 mg/ml. In another study, the aqueous and ethanolic extracts of *B. pinnatum* leaf were reported to show antibacterial activity against *P. aeruginosa*, and *S. aureus* isolated from patients with *otitis media* [25].

The difference in the extraction solvent may account for this disparity. Also, the variation in tested isolates, with MDR isolates in the current study versus non-MDR isolates in the previous study, could contribute to the differences.

The antibacterial activities demonstrated by the ethanolic leaf extract of *B. pinnatum* in this

study may be linked to the presence of phenolic compounds such as alkaloids, tannins, flavonoids, saponins, and steroids [26,27]. These phenolic compounds have previously shown antagonistic effects against both Gram-positive and Gram-negative human pathogens, including *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *S. typhi*, *P. aeruginosa*, and *Bacillus subtilis* [28].

MIC testing revealed that the extract inhibited growth of the isolate between concentrations of 100 and 200 mg/ml, suggesting a bacteriostatic effect against *S. typhi* and *Proteus* sp. MBC testing indicated bactericidal activity against *Proteus* sp at 200 mg/ml.

Conclusion

The antibacterial activities of *B. pinnatum* ethanolic leaf extract against MDR *E. coli*, *P. aeruginosa*, and *S. aureus* underscore the plant's potential for development in treating diseases caused by these pathogens. Therefore, further exploration of *B. pinnatum* is recommended for its potential as a source of antibacterial agents and raw materials for the pharmaceutical industry. Future research should delve into the specific antibacterial components and mechanisms of action for a more comprehensive understanding.

Acknowledgements

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Conflict of Interest

The authors declare no potential conflict of interest.

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