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ANTIBACTERIAL EFFICACY OF BRYOPHYLLUM PINNATUM LEAF EXTRACTS AGAINST COMMON PATHOGENIC BACTERIA: A COMPARATIVE IN VITRO STUDY

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ABSTRACT

The development of germs that are resistant to several drugs has made it necessary to investigate novel, safe, and efficient antimicrobials derived from natural sources. In traditional medicine, *Bryophyllum pinnatum*, a commonly used medicinal herb, has showed potential. This study examines the antibacterial efficacy of *B. pinnatum* leaf solvent extracts (acetone, methanol, chloroform, ethyl acetate, and aqueous) against four pathogenic bacteria: *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Escherichia coli*. To evaluate antibacterial efficacy, zones of inhibition were evaluated using the disc diffusion method. The strongest inhibitory activity was shown by acetone and aqueous extracts, especially against *S. typhi* and *E. coli.* These results point to *B. pinnatum's* potential as a natural antibacterial agent and validate its ethnomedical claims.

KEYWORDS: *Bryophyllum pinnatum*, Antimicrobial, *Escherichia coli*, Natural antibiotics, zone of inhibition, Disc diffusion assay.

INTRODUCTION

One of the biggest risks to public health worldwide in the twenty-first century is the rising incidence of antibiotic resistance in harmful bacteria. The World Health Organization (WHO) estimates that if current trends continue unchecked, resistant diseases will kill more than 10 million people a year by 2050.[1] The development of resistant strains has been sped up by the overuse and abuse of traditional antibiotics, making the search for sustainable and alternative antimicrobial sources imperative. In this regard, medicinal plants are a huge source of pharmacologically

active substances that have a great deal of promise for fighting bacterial infections. More than 80% of people in underdeveloped nations practice traditional medicine, which frequently uses plant-based treatments to treat infectious infections.[2] *Bryophyllum pinnatum*, a member of the Crassulaceae family, is one of these medicinal plants that has garnered a lot of interest because of its many therapeutic uses, which include anti-inflammatory, antioxidant, analgesic, antipyretic, wound-healing, and antimicrobial effects.[3,4] This succulent plant, which is native to Madagascar and has spread throughout tropical and subtropical countries, including India, is referred to as a "life plant" or "miracle leaf" because of its rich ethnopharmacological history and regenerating qualities.[5]

Numerous bioactive components, including flavonoids, alkaloids, phenolic compounds, bufadienolides, saponins, and tannins, have been identified by phytochemical investigations of *B. pinnatum*.[6,7] These substances are known to cause growth suppression or cell death by rupturing bacterial membranes, inhibiting enzymes, or interfering with the production of nucleic acids.[8] The antibacterial efficacy of several solvent extracts of *B. pinnatum* leaves against a range of bacterial strains, including both Gram-positive and Gram-negative pathogens, has been reported in earlier investigations.[9,10] Additionally, recent research has demonstrated that chemicals produced from plants can be used as a starting point for the creation of nanoparticles with improved antibacterial activity. For example, the green production of zinc oxide and silver nanoparticles using extracts from *B. pinnatum* has shown enhanced inhibitory effects against bacteria that form biofilms and multidrug-resistant (MDR) uropathogens.[11] These developments highlight the plant's ongoing significance and promise in the creation of innovative antibacterial agents.

The goal of this work was to assess the in vitro antibacterial activity of several solvent extracts of *B. pinnatum* leaves against *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Escherichia coli*, among other clinically relevant pathogens. The study intends to promote future research into creating herbal antibacterial formulations or adjuvants to traditional antibiotics, as well as to validate ethnobotanical claims.

MATERIALS AND METHODS

Plant Collection and Preparation

Fresh leaves of *Bryophyllum pinnatum* were collected from Maruthoorkurichi, Kanyakumari district, Tamil Nadu, India. Following washing, drying in the shade, powdering, and cold maceration, the leaves were extracted using solvents (methanol, acetone, chloroform, ethyl acetate, and distilled water). Evaporation was used to filter and concentrate the extracts.

Microbialstrains

Salmonella typhi, *Shigella dysenteriae*, *Escherichia coli*, and *Staphylococcus aureus* clinical isolates were employed. At 4°C, the bacterial strains were kept in nutritional broth.

Antibacterial Assay

The Kirby-Bauer disc diffusion method was used to evaluate the antibacterial activity. Every bacterial suspension was injected onto Mueller-Hinton agar plates. The inoculation plates were covered with sterile discs (5 mm) impregnated with each plant extract, which were then incubated for 24 hours at 37°C. Centimeters were used to measure the zones of inhibition.

RESULTS

Four bacterial pathogens were used to test the antibacterial activity of different solvent extracts of *Bryophyllum pinnatum* leaves: *Salmonella typhi, Escherichia coli, Shigella dysenteriae,* and *Staphylococcus aureus*. The diameter of the zone of inhibition surrounding the discs impregnated with each plant extract was used to evaluate the results, which are compiled in Table 1 and Figure 1. The acetone and aqueous extracts showed the strongest antibacterial activity among the studied extracts. Strong bacteriostatic or bactericidal capability against this Gram-negative pathogen was demonstrated by the acetone extract, which exhibited the biggest zone of inhibition (1.9 cm) against *E. coli*, closely followed by the aqueous extract (1.8 cm). Similarly, *Salmonella typhi* had a high susceptibility, especially to the aqueous extract, which demonstrated a considerable antibacterial effect with a maximal inhibition zone of 1.9 cm. While the ethyl acetate extract had a relatively low effect on *S. aureus* and *S. dysenteriae* (0.6 cm and 0.0 cm, respectively), it had significant activity, particularly against E. coli (1.5 cm) and S. typhi (0.9 cm). However, the bactericidal activity of methanol and chloroform extracts was inconsistent and frequently lower. While methanol extract was less efficient against *S. aureus* (0.2 cm), it produced moderate zones of inhibition against *S. typhi* (1.2 cm) and *S. dysenteriae* (0.7 cm).

Chloroform extract exhibited mild inhibition against *S. aureus* (0.9 cm) and *E. coli* (1.0 cm), and no discernible inhibition against *S. dysenteriae* or *S. typhi*. Overall, it seems that the bioactive components in B. pinnatum were more effective against Gram-negative bacteria, especially *E. coli* and *S. typhi*, than against Gram-positive *S. aureus*. This could be because the makeup of the cell walls of the two types of bacteria differs, which can alter the penetration of extracts. The fact that ethyl acetate and chloroform extracts showed no inhibition against *S. dysenteriae* highlights how pathogen-specific phytochemical effectiveness is. Solvent polarity is a crucial factor in the extraction of phytoconstituents with antibacterial qualities, as the comparative study of several extracts made abundantly evident. The highest antibacterial activity (up to 1.9 cm zone of inhibition) was shown by the acetone and aqueous extracts, particularly against *S. typhi* and *E. coli*. The activity of methanol, chloroform, and ethyl acetate extracts was moderate to mild.

Table 1: Zone of inhibition (cm) of *B. pinnatum* extracts against bacterial strains.

		Zone of inhibition (cm)				
Sl. No	**Pathogens**	**Acetone extract**	**Ethyl acetate extract**	**Methanol extract**	**Chloroform extract**	**Aqueous extract**
1	*Staphylococcus aureus*	1.3	0.6	0.2	0.9	0.8
2	*Shigella dysentriae*	1.3	0	0.7	0	1.2
3	*Salmonella typhi*	1.2	0.9	1.2	0	1.9
4	*Escherichia coli*	1.9	1.5	0.7	1.0	1.8

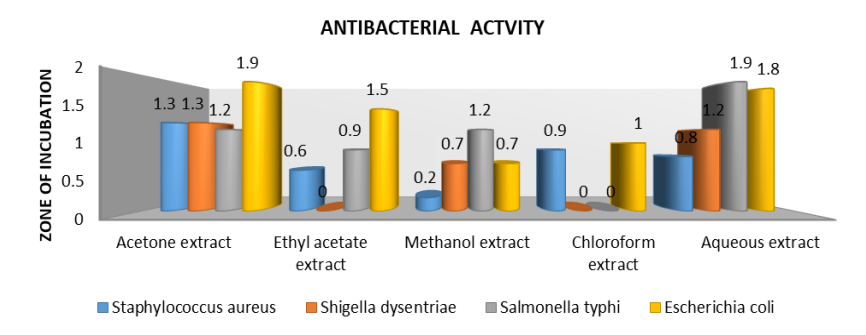

Figure 1: Antibacterial activity of various leaf extracts of Bryophyllum pinnatum.

DISCUSSION

The current study demonstrates that *Bryophyllum pinnatum* leaf extracts are effective against a number of clinically relevant infections. Acetone and aqueous extracts consistently shown the highest inhibitory effects among the five solvent extracts studied, especially against *Salmonella typhi* and *Escherichia coli*. These results are consistent with previous studies that revealed a higher concentration of bioactive phytochemicals, such as flavonoids, polyphenols, and glycosides with antimicrobial properties, were extracted by polar solvents including acetone and water.[12,13] The effective solubilization of mid-polar to non-polar phytocompounds, such as bufadienolides and phenolic acids, which can break down bacterial membranes and inhibit essential enzymes, may be the cause of the acetone extract's enhanced action (zone of inhibition: 1.9 cm) against E. coli.[14] A broad antibacterial spectrum has been indicated by similar studies on *Bryophyllum pinnatum* extracts, which have shown suppression of methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[15,16]

The high activity of the aqueous extract, particularly against *S. typhi* (1.9 cm), disproves previous theories that water is a bad solvent for removing non-polar antibacterial substances.[17] The existence of hydrophilic bioactives, such as organic acids, tannins, and polysaccharides, which can work alone or in concert to provide bacteriostatic effects, can help to explain this.[18] Aqueous extracts of *B. pinnatum* have been shown in recent investigations to exhibit strong suppression of uropathogenic strains and to disrupt quorum sensing pathways and bacterial biofilm formation.[19] Methanol and chloroform extracts' comparatively decreased efficacy indicates either a restricted extraction of important antibacterial components or potential antagonistic interactions between specific chemicals in those fractions.[20] Furthermore, the lack of activity against *Shigella dysenteriae* in certain ethyl acetate and chloroform samples emphasizes how phytochemicals' selective effectiveness is reliant on the permeability barriers and bacterial structure. The presence of particular targeted chemicals in the *B. pinnatum* extracts is probably why gram-negative bacteria, which are often more resistant because of their outer membrane, seemed more sensitive in our investigation.[21]

The notion that *B. pinnatum* exhibits antibacterial activity via a variety of pathways, such as bacterial cell wall breakdown, protein synthesis inhibition, and modification of nucleic acid metabolism, is further supported by recent pharmacological data.[22,23] It has been shown that certain fractions rich in bufadienolide block ATPase, which hinders the energy metabolism of bacteria.[24] Furthermore, by reducing host tissue damage and enhancing innate immune responses during infection, *B. pinnatum's* anti-inflammatory and antioxidant qualities may improve antimicrobial efficacy.[25] Improved antibacterial effectiveness and stability against MDR bacteria have been demonstrated by nanotechnology-based improvements employing silver or zinc oxide nanoparticles produced from *B. pinnatum*, underscoring its potential in next-generation phytopharmaceutical formulations.[26, 27] Sub-inhibitory quantities of plant extracts have been shown to lower the minimum inhibitory concentrations (MIC) of common antibiotics such as ciprofloxacin and amoxicillin, demonstrating the synergistic potential of combining *B. pinnatum* extracts with conventional antibiotics.[28] Validating traditional medicinal plants like *B. pinnatum* as a source of novel antimicrobial drugs is crucial and relevant given the growing global problem of antimicrobial resistance (AMR). The study's findings provide credence to its incorporation into alternative therapies, especially in areas with restricted access to synthetic medications.[29] To fully utilize its pharmacological potential, however, more investigation is needed, including bioassay-guided fractionation, active ingredient isolation, and molecular target elucidation.[30] To guarantee safety and effectiveness in medicinal applications, toxicity evaluations and clinical studies are required.

CONCLUSION

This study demonstrates the strong antibacterial activity of *Bryophyllum pinnatum* leaf extracts, especially those made with acetone and aqueous solvents, against clinically significant bacterial pathogens like *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. Strong inhibitory zones against *S. typhi* and *E. coli* indicate the presence of strong bioactive substances that need more pharmacological investigation. These results offer a scientific basis for the possible incorporation of *B. pinnatum* into contemporary antimicrobial treatments, in addition to validating its historic use in the treatment of illnesses. The significance of solvent selection in phytochemical extraction is further highlighted by the extracts' varying activities. The identification and creation of plant-based antimicrobials, like those in *B. pinnatum*, present viable, sustainable substitutes for traditional antibiotics in the face of growing antibiotic resistance. To fully realize this promise, more research is needed to identify the mechanisms of action of the active ingredients, isolate them, test their synergistic interactions with conventional antibiotics, and perform toxicity and clinical safety evaluations. In the end, *B. pinnatum* may prove to be a useful natural resource for the creation of innovative antimicrobial compounds in the nutraceutical and pharmaceutical sectors.

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