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In Vitro Bactericidal Activity of *Bacopa Monnieri* Leaf Extracts on Human Pathogenic Bacteria

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Abstract: The increasing resistance of bacteria to conventional antibiotics poses a global health challenge, emphasizing the urgent need to discover new antibacterial compounds to combat hard-to-treat infections. This study aims to evaluate the bactericidal activity of crude leaf extracts from the multi-medicinal plant *Bacopa monnieri* against six bacterial strains. The crude extracts, prepared at different concentrations (100, 200, and 300 µg/mL), were tested for efficacy using the agar well diffusion method. Among the tested extracts, the ethanol extract exhibited the highest bactericidal activity, followed by chloroform and hexane extracts. Notably, all three solvent extracts at 300 µg/mL were most effective against *Staphylococcus aureus*, with inhibition zones of 21.5 mm (ethanol), 16.12 mm (chloroform), and 10.3 mm (hexane). In contrast, the lowest antibacterial activity was observed against Proteus vulgaris, with inhibition zones of 15.3 mm (ethanol), 10.2 mm (chloroform), and 5.6 mm (hexane). These findings suggest that the ethanol extract of *B. monnieri* is a promising candidate for the development of novel antibacterial compounds.

Introduction

Herbal medicines are important sources of novel compounds with pharmaceutical applications. The present-day pharmacological experiments have led to the use of herbal medicines for complementary and alternate therapy. To date, about 80% of the people in developing countries use traditional medicines (1). The majority of human diseases up to 30% have been caused by bacterial strains, including *Bacillus subtilis, Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (2, 3). Several synthetic antibacterial agents have been available for human pathogenic bacteria, but their usage is limited since they are toxic and also expensive. However, plant-based medicines are relatively safer than synthetic drugs, which offer very good therapeutic benefits and are also affordable (4-5).

Bacopa monnieri (L.) Pennell is an endangered multi-medicinal plant belonging to the family Scrophulariaceae. In India, it is popularly known as 'Brahmi' and is used in Ayurveda for the preparation of medhyarasayan, which is a drug used to improve intelligence and memory (6). *B. monnieri* is distributed throughout the country generally its habitat is wet, damp, and marshy places. It is used for the treatment of various ailments, such as antipyretic, antiinflammatory, analgesic, asthma, epilepsy, insanity, anticancer, antioxidant activities, blood clearing, hoarseness, memory enhancement, and water retention (7-13). It has a wide variety of secondary metabolites such as Alkaloids (Nicotine, Brahmine, and Herpestine); Terpenoids (Tetracyclic terpenoids (bacoside A, B, C and D); Stigmasterol; β-Sitosterol); Flavonoids (Luteolin; Apigenin; Quercetin) and Phenolic compounds (tannins) which have been reported (14-17).

The relationship between plant secondary metabolites and antimicrobial activity has been well described by Cowan (18). Given the medicinal importance of *B. monnieri*, various studies have investigated its antimicrobial properties using different solvent extracts (19-31). However, reports on the

bactericidal activity of *B. monnieri* leaf extracts remain limited. Considering its role in traditional medicine and therapeutic applications (32), this study focuses on evaluating the bactericidal activity of hexane, chloroform, and ethanol extracts from *B. monnieri* leaves.

Materials and Methods

Collection of Plant Material

The medicinal herb *B. monnieri* was collected from the Horticulture Division, Andhra University, India, with specimen no. AUV24002. The leaf material was carefully gathered, thoroughly washed with water, and then dried in the shade. The air-dried leaves were ground using a mortar and pestle to obtain a fine leaf powder.

Preparation of Solvent Extracts

The leaf powder of *B. monnieri* up to 10 g was taken and packed in Soxhlet apparatus then the solvent was added in a round bottom flask and was heated over a heating mantle using the adjustable rheostat. The extraction was done for up to 72 h separately with solvents hexane, chloroform, and ethanol. Then the individual solvent extract was collected and concentrated by evaporating the respective excess solvent.

Preparation of Nutrient Agar Medium

The components required for the nutrient agar medium, namely peptone (0.5 g), beef extract (0.3 g), and sodium chloride (0.5 g), were added to a conical flask and dissolved in 100 mL of distilled water. The pH was adjusted to 7.2, followed by the addition of 1.5 g of agar-agar. The mixture was then sterilized in an autoclave at 121 °C under 15 lbs of pressure for 15–20 min.

Microbial Cultures

Six bacterial strains were obtained from the Microbial Culture Collection (MTCC) of IMTECH, Chandigarh, India, for antibacterial activity testing. These included three Gram-positive strains (*Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*) and three Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*).

Preparation of Microbial Pure Cultures

Pure cultures of all six bacterial strains were prepared on nutrient agar medium by inoculating a loopful of stock culture onto nutrient agar plates, followed by incubation at 37 °C overnight. The bacterial cultures were maintained on nutrient agar plates and stored at 4 °C.

Antimicrobial Assay

The crude hexane, chloroform, and ethanol leaf extracts of *B. monnieri* were screened for their antibacterial activity using the agar well diffusion method, as described by Collins *et al*. (33). First, nutrient agar plates were inoculated with different strains of test bacteria. Then, three wells (6 mm in diameter) were made in each plate using a sterile cork borer. Different concentrations of crude extracts (100, 200, and 300 µg/mL) were added to the respective wells. Separate plates containing 10 µg/mL ciprofloxacin and 0.1% dimethyl sulfoxide (DMSO) were prepared as positive and negative controls, respectively.

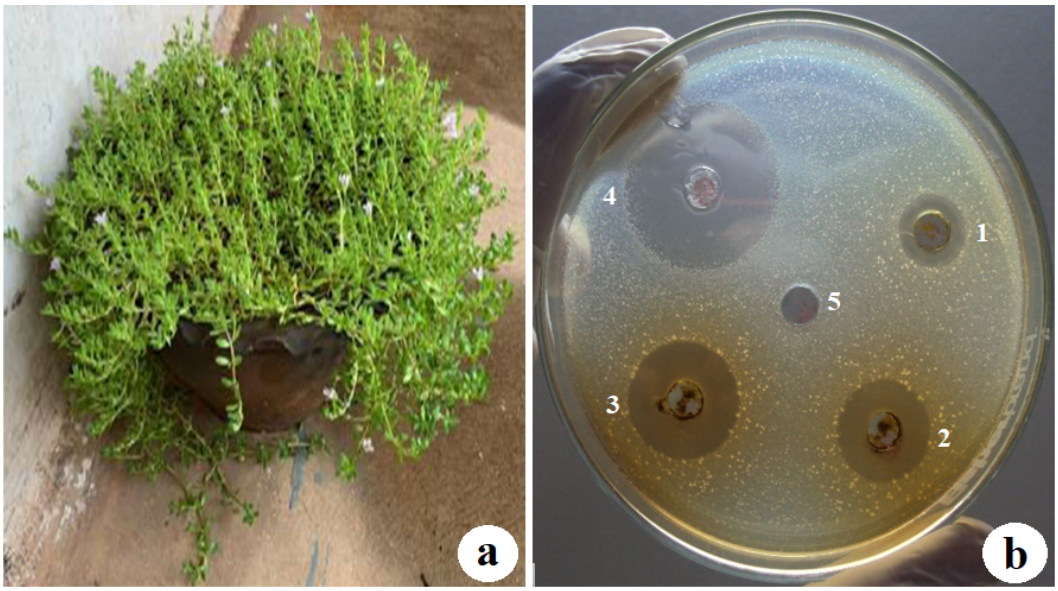

Figure 1. Bactericidal activity of *Bacopa monnieri* (L.) Pennell: a) *Bacopa monnieri* in a garden pot; b) crude leaf ethanol extract antimicrobial activity on *Staphylococcus aureus.* **Note:** 1-3: Inhibition zones of crude leaf ethanol extracts con.100; 200; 100µg/ml respectively; 4: positive control zone of inhibition; 5: negative control zone of inhibition.

Table 1. Invitro bactericidal activities of crude leaf extracts of *Bacopa monnieri*.

No.	Microorganism	Inhibition zone (mm)		
		100 µg/ml	200 µg/ml	300 µg/ml
n-Hexane extract				
1.	*Bacillus subtilis*	2.10 ± 0.12	4.00 ± 0.15	8.40 ± 0.22
2.	*Staphylococcus aureus*	2.50 ± 0.24	4.80 ± 0.30	10.30 ± 0.23
3.	*Enterococcus faecalis*	2.30 ± 0.22	4.60 ± 0.25	9.30 ± 0.20
4.	*Escherichia coli*	2.20 ± 0.15	3.60 ± 0.30	8.00 ± 0.33
5.	*Pseudomonas aeruginosa*	2.00 ± 0.27	3.20 ± 0.26	7.60 ± 0.40
6.	*Proteus vulgaris*	1.90 ± 0.35	2.80 ± 0.22	5.60 ± 0.23
Chloroform extract				
1.	*Bacillus subtilis*	4.10 ± 0.18	6.50 ± 0.20	14.00 ± 0.28
2.	*Staphylococcus aureus*	4.30 ± 0.23	7.80 ± 0.27	16.12 ± 0.26
3.	*Enterococcus faecalis*	3.80 ± 0.18	7.10 ± 0.32	15.20 ± 0.28
4.	*Escherichia coli*	3.50 ± 0.26	6.50 ± 0.24	13.10 ± 0.28
5.	*Pseudomonas aeruginosa*	3.15 ± 0.30	5.20 ± 0.38	12.80 ± 0.33
6.	*Proteus vulgaris*	3.10 ± 0.19	5.90 ± 0.30	10.20 ± 0.21
Ethanol extract				
1.	*Bacillus subtilis*	5.90 ± 0.23	12.40 ± 0.20	17.18 ± 0.22
2.	*Staphylococcus aureus*	6.50 ± 0.32	14.70 ± 0.25	21.50 ± 0.28
3.	*Enterococcus faecalis*	6.10 ± 0.27	13.30 ± 0.32	19.80 ± 0.31
4.	*Escherichia coli*	5.30 ± 0.18	11.10 ± 0.29	16.50 ± 0.26
5.	*Pseudomonas aeruginosa*	4.80 ± 0.26	10.20 ± 0.19	15.60 ± 0.31
6.	*Proteus vulgaris*	4.30 ± 0.32	8.60 ± 0.27	15.30 ± 0.24
		Negative control		**Positive control**
1.	*Bacillus subtilis*	0.00 ± 0.00		35.00 ± 0.29
2.	*Staphylococcus aureus*	0.00 ± 0.00		36.00 ± 0.32
3.	*Enterococcus faecalis*	0.00 ± 0.00		35.80 ± 0.30
4.	*Escherichia coli*	0.00 ± 0.00		25.00 ± 0.20
5.	*Pseudomonas aeruginosa*	0.00 ± 0.00		24.50 ± 0.22
6.	*Proteus vulgaris*	0.00 ± 0.00		24.50 ± 0.26

All plates were left at room temperature for 15–20 min to allow the extracts to diffuse into the medium before being incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the diameter of the inhibition zones. All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation.

Results and Discussion

Since ancient times, medicinal plants have been used as herbal medicine due to their therapeutic value. They are valuable sources of bactericidal compounds that can be used to treat diseases caused by bacteria and other pathogenic microorganisms. Nature provides a vast array of plants whose extracts contain numerous secondary metabolites with antibacterial, antiviral, antifungal, and antioxidant properties, making them essential in treating various diseases. The appearance of *B. monnieri* and the antimicrobial effects of its extract can be seen in **Figure 1**.

The bactericidal activity of different crude extracts—hexane, chloroform, and ethanol—from the leaf extract of *B. monnieri* exhibited significant inhibition against six human pathogenic bacterial strains. The results were expressed as the diameter of the inhibition zone for each crude solvent extract at three different concentrations (100, 200, and 300 µg/mL) against the tested bacteria, as presented in **Table 1**. All solvent extracts of B. monnieri demonstrated remarkable antibacterial activity, indicating that the crude leaf extracts contain various antibacterial agents that could be used to treat infectious diseases caused by different pathogenic bacteria.

Among the tested extracts, the ethanol extract exhibited the highest antibacterial activity against both

Gram-positive and Gram-negative bacteria compared to the other extracts. In this study, the antibacterial efficacy of the crude solvent extracts followed the order: ethanol > chloroform > hexane. Additionally, the highest antibacterial activity was observed at a concentration of 300 µg/mL, followed by 200 and 100 µg/mL. The negative control (DMSO) showed no inhibitory effect, whereas the positive control (ciprofloxacin) exhibited the largest inhibition zones against all tested bacterial strains.

The ethanol extract showed a significant inhibition of bacterial growth, with inhibition zones ranging from 4.30 mm to 21.50 mm. The maximum inhibition zone (21.50 ± 0.28 mm) was recorded at a concentration of 300 µg/mL against Staphylococcus aureus, while the minimum inhibition zone (4.30 ± 0.32 mm) was observed at 100 µg/mL against Proteus vulgaris. These findings align with previous reports, with slight variations (34-36).

The crude chloroform extract demonstrated moderate antibacterial activity, with inhibition zones ranging from 3.10 mm to 16.12 mm, consistent with the observations of Ayyapan *et al*. (37). However, Kalaivani *et al*. (24) reported that the chloroform extract of *B. monnieri* exhibited lower activity against Gram-positive bacteria and no activity against Gram-negative bacteria.

The lowest bactericidal activity was observed with the hexane extract, with inhibition zones ranging from 1.90 mm to 10.30 mm. These results are supported by the findings of Mathur *et al*. (23), Basak *et al*. (38), and Verma and Kumar (39). The present study also indicates that Gram-positive bacteria are more sensitive to *B. monnieri* extracts than Gram-negative bacteria, which aligns with previous studies on Bacopa's antibacterial activity (21, 23, 26, 29). This difference in susceptibility may be attributed to variations in bacterial cell wall composition (40, 41).

Among the tested bacteria, *Staphylococcus aureus* was the most sensitive, while *Proteus vulgaris* was the most resistant. Based on these findings, the crude solvent extracts of *B. monnieri* leaves contain specific bactericidal compounds. Therefore, further studies are recommended to identify and characterize the active chemical compounds responsible for this antibacterial activity.

Conclusion

The three solvent leaf extracts of *B. monnieri* at different concentrations (100, 200, and 300 µg/mL) exhibited varying degrees of bactericidal activity against the tested microorganisms. Among these concentrations, 300 µg/mL was the most effective, with inhibition zones ranging from 15.3 to 21.5 mm for ethanol extract, 10.2 to 16.12 mm for chloroform extract, and 5.6 to 10.3 mm for hexane extract.

Among the three solvent extracts, the ethanol extract demonstrated the highest antibacterial activity against all tested bacterial strains, suggesting its potential as a source of new antibacterial agents. Further studies on the phytochemical and pharmacological properties of the ethanol leaf extract of *B. monnieri* may help identify bioactive compounds for the treatment of bacterial infections.

Declarations

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Conflict of Interest

The author declares no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

Funding Information

Not applicable.

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