

## Antibacterial efficacy of the leaves of the medicinal plant *Bacopa monnieri*

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### ABSTRACT

*Bacopa monnieri* also known as “Neer-Brahmi” has been considered an important medicinal plant in Ayurvedic systems for centuries. The present study was carried out to evaluate the antimicrobial potential of the aqueous leaf extract of *B. monnieri* (L.). Four different concentrations (25, 50, 75 and 100 µl/ml) of leaf extract of *B. monnieri* (L.) were tested for antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumoniae* bacterial strains. The sensitivity of plant fractions was tested using the disc diffusion method. The minimum inhibitory concentration (MIC) of leaf extract was evaluated against each bacterium. Leaf extract demonstrated effective activity against the tested bacteria, with inhibition zone diameters ranging from 6.08-17.83 mm and their efficacy in terms of MICs ranged from 1.56 to 6.25 µl/ml. In conclusion, the results indicate that the *B. monnieri* leaf extract contains essential metabolites in the search for new effective antibacterial agents.

**Keywords:** *Bacopa monnieri*, Antibacterial Activity, Disc Diffusion Assay, Minimum Inhibitory Concentration

### 1. Introduction

Infectious diseases have been recognized as one of the major intimidations to human health throughout the world. Most of them are caused by microorganisms such as bacteria, viruses, and fungi [1]. It is reported that bacteria are attributed to approximately 30% of all diseases, leading to millions of deaths every year [2]. The development of bacterial resistance against available antibiotics has necessitated the need to search for new antibacterial agents.

Active compounds with antibacterial activity have been identified in plants so far to develop new promising drugs. Compared with synthetic drugs, plant-based antibiotics are considered to be safer due to their natural origin. Plants can produce a variety of organic chemicals of high structural diversity, called secondary metabolites, such as alkaloids, terpenoids, flavonoids, and tannins, which have antimicrobial properties *in vitro* [3]. The most important advantages claimed for the use of herbal plants in various diseases are their safety, in addition to being economical, effective, and easily available [4].

*Bacopa monnieri* (L.) commonly known as Neer-Brahmi, belonging to the family Scrophulariaceae is a creeping small prostrate annual herb growing widely in tropical regions of Asia [5]. It is mostly used in Ayurvedic medicine for treating various diseases like ulcers, tumors, ascites, indigestion, enlarged spleen, leprosy, inflammations, anemia, and biliousness. Therefore, the purpose of the present study was to screen the antibacterial activity of *Bacopa*

*monnieri* leaf extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

## 2. Materials and Methods

**Sample Collection:** The leaves of *Bacopa monnieri* were collected from the Western Ghats, approximately located at 10°10'N 77°04'E in Tamil Nadu, India. Fresh leaves were cleaned several times with distilled water and dried at room temperature in the open air for further analysis.

**Sample Preparation and Extraction:** The leaves were air-dried at room temperature, ground to powder with a mechanical grinder to obtain a fine powder and stored in an air-tight container for further use. 2.5 g of *Bacopa monnieri* leaves powder is mixed with 50 ml of distilled water and kept in a water bath for 30 minutes. The colour change in the mixture from light green to brownish colour implied that the extraction has occurred. The extract was filtered through Whatmann No.1 to remove any undissolved materials. The obtained solution was stored in clean containers at 4°C for further analysis.

**Bacterial culture:** The test microorganisms *Staphylococcus aureus* (MTCC 916) and *Klebsiella pneumoniae* (MTCC 503) were purchased from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

### Antibacterial activity

**Disc diffusion method:** For the disc diffusion assay, the plates were swabbed with pathogenic bacterial culture viz. *Staphylococcus aureus* and *Klebsiella pneumoniae* was evenly swabbed on a solidified 25 ml MHA. Discs (6 mm in diameter) were punched from a sheet of Whatmann filter paper, sterilized, and impregnated with different concentrations of leaf extract (25 µl, 50 µl, 75 µl, and 100 µl). Thereafter, the discs were placed on the surface of inoculated MHA plates and then incubated at 37°C for 24 h to observe the formation of inhibition zones around the discs. The size of the zone of inhibition (including the disc) was measured in millimeters.

**Determination of minimum inhibitory concentration (MIC):** Minimum inhibitory concentration (MIC) is the lowest concentration of the drug which will inhibit growth as measured by observed turbidity in the test tube [6]. The broth micro dilution method was used to determine the MIC. *B. monnieri* leaf extract was dissolved in 10% DMSO and diluted to a higher concentration. Then, serial ½ dilutions of leaf extract were prepared directly in a microtiter plate containing Mueller Hinton broth to obtain concentrations from 0.78 to 50 µl/ml. The bacterial inoculum was added to give a final concentration of  $5 \times 10^5$  CFU/mL in each well. The positive control was used containing Gentamicin as the standard drug at final concentrations from 0.125 to 128 µl/ml. The plate was covered with a sterile sealer and

incubated for 24 h at 37°C.

**Statistical Analysis:** The experimental results were expressed as mean  $\pm$  standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by post hoc tukey test. P values less than 0.05 were considered statistically significant. Microsoft Excel 2010 statistical package was used for all analyses.

### 3. Results

In the present study, the antibacterial efficacy of *B. monnieri* leaf extract was evaluated against two bacterial species *Staphylococcus aureus* and *Klebsiella pneumoniae* by disc diffusion method. By employing the Kirby Bauer well diffusion technique antibacterial activity of different concentrations of leaf extract viz. 25  $\mu$ l, 50  $\mu$ l, 75  $\mu$ l, 100  $\mu$ l, plain disc and Streptomycin is corroborated to perform a comparative study and obtained results are given in Table 1. Fig.1 confers the Zone of inhibition (in mm).

The antibacterial activity was clearly expressed in terms of a clear zone of inhibition of the leaf extract. *Bacopa monnieri* leaf extract showed inhibition zones in all the concentrations against the two bacterial strains. The zone of inhibition increases as the concentrations of extract increase in both the tested bacterial strains.

Aqueous extract of *B. monnieri* leaf extract recorded the lowest antibacterial activity against *S. aureus* with an inhibition zone of 6.08 mm at the concentration of 25  $\mu$ l while the highest activity was observed at the concentration of 100  $\mu$ l against *K. pneumoniae* with 17.83 mm inhibition. The highest level of inhibitory effect was seen at the concentration of 100  $\mu$ l for *S. aureus* and *K. pneumoniae* showing 12.9 mm and 17.83 mm antibacterial activity. When compared statistically, the difference in mean zone of inhibition between *S. aureus* and *K. pneumoniae* was nonsignificant (p value- 0.13 > 0.05).

The results of the present study revealed that the inhibitory effect of *B. monnieri* against *K. pneumoniae* was near to the standard drug Streptomycin. While comparing the inhibitory rates of both the tested organisms, the growth of Gram-negative *K. pneumoniae* was more effectively inhibited than the Gram-positive *S. aureus*.

**Table 1. Inhibitory activity of *B. monnieri* leaf extract on bacteria**

Concentration ( $\mu$ l/ml)	Zone of inhibition (mm)		
	<i>Staphylococcus aureus</i> (G+)	<i>Klebsiella pneumoniae</i> (G-)	Streptomycin (Control)
25 $\mu$ l	6.08 $\pm$ 0.62	8.82 $\pm$ 0.27	9.59 $\pm$ 0.91
50 $\mu$ l	8.01 $\pm$ 0.49	12.5 $\pm$ 0.5	14.59 $\pm$ 0.5

75 $\mu$ l	10.33 $\pm$ 0.63	15.4 $\pm$ 0.61	18.09 $\pm$ 0.15
100 $\mu$ l	12.9 $\pm$ 0.13	17.83 $\pm$ 0.25	19.94 $\pm$ 0.78



**Fig 1. Antibacterial activities of *B. monnieri* leaf extract at different concentrations and Streptomycin as standard**

The effectiveness of the extracts on tested bacterial strains was determined by measuring the minimum inhibitory concentration (MIC) (Table 2). The minimum inhibitory concentration (MIC) was studied on *B. monnieri* leaf extract using different concentrations against *S. aureus* and *K. pneumoniae* bacteria. The results showed that leaf extracts could inhibit the growth of the two tested bacteria but with different sensitivities. MIC values of *B. monnieri* leaf extract were 6.25  $\mu$ l/ml against *S. aureus* and 1.56  $\mu$ l/ml against *K. pneumoniae*. The MIC of streptomycin against both the tested bacteria was 0.78  $\mu$ l/ml.

**Table 2. Antimicrobial activity expressed as Minimum Inhibitory Concentration (MIC  $\mu$ l/ml) of the *B. monnieri* leaf extract**

Bacterial name	MIC
<i>Staphylococcus aureus</i>	6.25 $\mu$ l/ml
<i>Klebsiella pneumoniae</i>	1.56 $\mu$ l/ml
Streptomycin (Control)	0.78 $\mu$ l/ml

#### 4. Discussion

Increasing the number of multi-drug resistance pathogenic microbes in humans and animals as well as unwanted side effects of certain antibiotics has encouraged enormous

interest to search for new antimicrobial drugs of plant origin [7]. The prime objective of ethnopharmacology is to identify plants of medicinal importance with minimal side effects. Additionally, active compounds from the plant extracts with antibacterial activity can be transformed into possible medication. Research to develop efficient and accessible medication from active plant compounds in the interest of public health is a need of the present world.

The present investigation was carried out to analyze the antibacterial efficacy of *B. monnieri* leaf extract against G+ve *S. aureus* and G-ve *K. pneumoniae*. From the results, it was clear that as the concentration increased, the zone of inhibition also increased in both bacterial species. *S. aureus* was less affected by *B. monnieri* leaf extract compared with *Klebsiella pneumoniae* even in high concentrations. Streptomycin has shown higher efficacy against *K. pneumoniae* as compared to *S. aureus*.

*B. monnieri* leaf extract showed different levels of inhibition depending upon the Gram-positive and Gram-negative bacteria. This might be due to the high antibiotic resistance of Gram-negative bacteria. Most Gram-positive bacteria such as *S. aureus* are surrounded by a coarse peptidoglycan cell wall. This structure, although mechanically strong, appears to offer little resistance to the diffusion of small molecules such as antibiotics [8]. *K. pneumoniae*, in contrast, as Gram-negative bacteria, surround themselves with a second membrane, the outer membrane, which functions as an effective barrier.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after 18–24 h. It was observed that against *S. aureus* and *K. pneumoniae*, *B. monnieri* leaf extract exhibited the minimum inhibitory values of 6.25 and 1.56 µl/ml as MIC respectively, which is a demonstration of a strong antimicrobial activity against both organisms. This result indicates that at a lower concentration, aqueous *B. monnieri* leaf extract is more effective against gram-negative bacteria than against gram-positive bacteria. This could be due to the complete solubility of the bioactive components of the plant in the extraction solvent and the inability of the cell membrane to exhibit a permeability barrier [9,10].

## 5. Conclusion

In this study, the aqueous leaf extract of *B. monnieri* was assessed for its antibacterial activity against *S. aureus* and *K. pneumoniae*. The results indicated that the leaf extract of *B. monnieri* had demonstrated significant antibacterial effects on bacterial strains tested, especially *K. pneumoniae*. This was confirmed by the determination of both diameters of inhibition zones and minimal inhibitory concentrations. This indicated that the *B. monnieri* plant has potent antibacterial properties and could be used in the development of novel antibacterial agents.

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