

Original Research Article

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## *Bacopa monnieri* L “Brahmi”: An Ayurvedic Herb with Promising Antibacterial Properties and its Future Application in Oral Hygiene

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### ABSTRACT

The investigation explores the characteristics of *Bacopa monnieri*, a renowned herb in Ayurvedic medicine referred to as 'Brahmi' for its wide-ranging applications in pharmacology, cosmetics, nutraceuticals, etc., showcasing efficacy against ulcers, inflammation, and asthma. The present study explores the antibacterial potential of *B. monnieri* for oral hygiene, particularly its impact on dental caries causing microorganisms, demonstrating favorable outcomes in both traditional and modern medical practices, offering cognitive enhancement and overall health benefits. The research aims to assess the antibacterial effectiveness against oral bacteria (*Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) utilizing the agar cup/plate technique by testing six different concentrations of methanolic extract derived from *Bacopa monnieri* (L.) Pennell. The results indicate that *Bacopa monnieri* Methanolic extract (BmMe) exhibits significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, with a concentration-dependent response observed in the zone of inhibition (ZOI) diameters, supported by the presence of various bioactive compounds identified through phytochemical screening, suggesting its potential as a natural antimicrobial agent and encouraging further exploration for incorporating it into mouthwash formulations as an innovative oral hygiene practices.

#### Keywords

Medicinal herbs,  
*Herpestis monnieri*  
L, *Scrophulariaceae*,  
Ayurvedic medicine

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### Introduction

Medicinal herbs have garnered significant attention and acknowledgment for their safety, efficacy, and noteworthy contributions to modern pharmacology. It is also important to highlight that plant extracts offer a potential reservoir of antimicrobial agents, providing affordable and effective herbal remedies for treating various disorders and diseases caused by common

bacterial infections (Mkk *et al.*, 2019). *Bacopa monnieri* (Linnaeus) Pennell. Synonym *Herpestis monnieri* L. family *Scrophulariaceae*, characterized as a creeping succulent herb, occupies a distinguished position as a medicinal plant with global recognition (Khan *et al.*, 2010; Tamboli *et al.*, 2022). Referred to as Brahmi in the realm of Ayurvedic medicine, *B.monnieri* has been harnessed for centuries to address a myriad of health conditions ranging from insanity, epilepsy, to skin

diseases (Mkk *et al.*, 2019; Tamboli *et al.*, 2022). The adaptability of this plant is evident through its manifestation of a diverse array of pharmacological activities encompassing sedative, vasoconstrictor, antimicrobial, antifungal, anti-inflammatory, antiepileptic, and anthelmintic properties (Mkk *et al.*, 2019).

Within *B. monnieri* also known as “the herb of grace,” lies a constituent named 'Bacosides', a triterpenoid saponin, which is credited for its memory-enhancing powers (Figure 1). The effects of Bacosides extend to the enhancement of nerve impulse transmission efficiency, thereby bolstering memory and cognitive functions (Mehta *et al.*, 2022). Moreover, this herb is employed as a remedy for ulcers, inflammation, anemia, scabies, leucoderma, epilepsy, and asthma, anticancer, antioxidant and antistress in addition to its role as a laxative (Khan *et al.*, 2010; Tamboli *et al.*, 2022).

In Ayurveda, *B. monnieri* is considered a brain tonic ‘*Medhya Rasayan*’ that enhances mental functioning and promotes overall health. The plant is known for its bitter taste, diuretic properties, blood purifying abilities, and enhancement of sexual vigor. It is also believed to improve urinary disorders. *Atharvaveda* mentions that *B. monnieri* strengthens the body and enhances the quality of semen. *Charak Samhita* refers to it as a life-sustaining tonic with invigorating properties, according to Ayurvedic principles (Verma, 2014). Collectively, *Bacopa monnieri* emerges as a multifaceted medicinal herb with a rich historical backdrop of therapeutic uses, notably acclaimed for its cognitive enhancements and diverse pharmacological properties. Jeddy *et al.*, (2018) conducted an experiment wherein they deduced that an herbal mouthwash exhibits higher efficacy in the reduction of oral bacteria in comparison to alternative chemical mouth rinses, thereby fostering safe and efficient oral hygiene practices (Jeddy *et al.*, 2018).

Consequently, the aim of the present investigation was to assess and scrutinize the antibacterial properties of *B. monnieri* extracts against a variety of tested bacterial strains and its potential prospective application as an active ingredient in oral rinses due to its wide-ranging pharmacological advantages in managing conditions like cough, asthma, inflammation, ulcers, or stress thereby presenting a notable progression for the healthcare sector. The analysis additionally encompassed the recognition and examination of plant chemicals in *Bacopa monnieri* (Tamboli *et al.*, 2022; Verma, 2014).

Dental caries, a pathological condition, arises due to the presence of particular bacterial strains, notably *Streptococcus mutans* and *Lactobacilli*, leading to the deterioration of dental structure (Teh *et al.*, 2015). Other microorganisms like *Streptococcus sobrinus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida spp.* have also been linked to the onset of dental caries (Cruz *et al.*, 2022; Teh *et al.*, 2015). In a conducted investigation by Cruz *et al.*, (2022), an analysis was carried out to assess the oral health status and identification of potentially pathogenic non-oral microorganisms like *Escherichia coli*, *Pseudomonas spp.*, *Candida albicans*, and *Staphylococcus aureus* among individuals hospitalized.

The outcomes of the study displayed a significant presence of microorganisms correlated with the spread of nosocomial infections and resistance to antimicrobial treatments within the oral cavity, indicating a sustained elevated abundance that the oral cavity has the potential to serve as a reservoir for clinically significant pathogens (Cruz *et al.*, 2022).

## Materials and Methods

### Source of Plant Material

*Bacopa monnieri* L. (Brahmi) powder was obtained from D.G & Sons Ayurvedic House of Herbs, located at 14 J.P. Road, Andheri West, Mumbai, India. The powder was crafted using freshly harvested aerial parts (leaves, stems) as well as terrestrial parts (roots) of the plants, which were carefully dried under the sun and then finely ground into powder form.

### Preparation of extract

A quantified amount of 20 grams of desiccated powder was soaked in 100 ml of methanol within a 250 ml flask in two batches each under ambient conditions for a duration of 24 hours. Each resultant solution was then filtered through Whatman filter paper no 1 and gathered in individual 100 ml beakers upon completion of the extraction process. The resulting filtrate was maintained at standard temperature and pressure until the extract had completely dried/desiccated.

The mass of the desiccated extract was recorded as 3.33 grams, resulting in a yield of the extract at 16.65% (w/w). Subsequently, the extract was preserved in airtight container at 4°C for further analyses (Mkk *et al.*, 2019).

## Microorganisms tested

The extracts of *B. monnieri* were tested for possible antibacterial activity using agar cup/plate against two Gram positive bacteria namely *Streptococcus mutans* and *Staphylococcus aureus* and two Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*. The bacterial strains were pure and obtained by the courtesy of the Department of Microbiology, Bhartiya Vidya Bhavan's College, Andheri (W) Mumbai and was maintained at 4°C on Nutrient agar slants.

## Culture Media used

The antibacterial test was carried out on Muller Hinton Agar (MHA) for *E.coli*, *S.aureus*, *P.aeruginosa* and Brain Heart Infusion Agar (BHI) for *S.mutans*.

## Drug used

An antibiotic disc containing Amikacin at a concentration of 30 ug/ml was employed as the positive control standard for the evaluation of any resistance profiles of the four examined microorganisms *E.coli*, *P.aeruginosa*, *S.aureus* and *S.mutans*.

## Preparation of sample solutions

0.1 gram of solid methanolic extract of Brahmi was quantified and subsequently dissolved in a specific volume (10 ml) of Dimethyl Sulfoxide (DMSO) to yield a solution with a concentration of 10 mg/ml. The selection of DMSO as the solvent was based on its non-inhibitory influence on bacterial cultures, along with its remarkable ability to fully dissolve solid samples (Emran *et al.*, 2015; Mehta *et al.*, 2022). From the Standardized stock solution of 10 mg/ml, various concentrations of the extract ranging from 1 mg/ml to 6 mg/ml were meticulously prepared using DMSO as a suitable diluent for achieving proper solubility. These selected concentrations were specifically chosen as the optimal ranges at which the antimicrobial assay was conducted under controlled laboratory conditions.

## Antibacterial activity assay

The determination of antibacterial activity was conducted through the agar cup/plate method (Mandal *et al.*, 2007). The assay plates containing the organisms *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. mutans* were prepared.

Each plate was inoculated with 1 ml fresh 24 hours grown culture of 0.2 optical density (OD) at 530 nm of the respective organism, poured with suitable media this resulted in the creation of two plates for each test organism. Subsequently, the plates were allowed to solidify over a period of about 5 to 10 minutes to ensure proper growth medium consistency.

Following solidification, a sterile cork borer was used under aseptic conditions to create wells with a diameter of 6 mm, with a total of 6 wells made for each organism across two separate plates (3 on each). Following this, 80 microliters (ul) of each sample, ranging in concentration from 1 mg/ml to 6 mg/ml, were carefully loaded into their respective wells. The prepared plates were then placed in an incubator set at 37 °C for a duration of 24 hours to allow for bacterial growth and the assessment of antibacterial activity. Next day the zone of inhibition (ZOI) was reported in millimeters (mm) for all the respective organisms and test organisms.

## Phytochemicals Analysis

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocol (Das *et al.*, 2014; Kancherla *et al.*, 2019; Theeba and Sasi Kumar, 2015). The results are summarized in Table 3 after conducting the following test.

### Test for alkaloids

Wagner's Test- 2 ml of extract was treated with wagner's reagent (1.27 gm Iodine + 2 gm Potassium iodide in 5 ml distilled water mix & make it 100 using distilled water) and formation of reddish brown precipitate indicates the presence of alkaloids.

### Test for flavonoids

NaOH Test- 2 ml of extract was treated with few drops of aqueous NaOH and HCl and formation of yellow orange color indicates the presence of flavonoids.

### Test for tannins

Ferric Chloride Test- Few ml of extract was added with alcohol and treated with neutral 5% ferric chloride solution and observed for formation of blue or greenish color solution indicates the presence of tannins.

### Test for saponins

Foam Test- A small amount of extract was shaken vigorously with water and the formation of persistent foam indicates the presence of saponins.

### Test for Terpenoids

Salkowski test- 5ml Extract was mixed with 2 ml chloroform, and 3 ml concentrated sulphuric, reddish brown color of interface indicates the presence of terpenoids.

### Test for Sterols

Liebermann- Burchard Test: 1 ml of extract was treated with chloroform, acetic anhydride and few drops of  $H_2SO_4$  was added and observed the formation of dark pink or red color or reddish brown ring indicates the presence of sterols.

### Test for Anthocyanin

NaOH Test- 2 ml of extract was treated with 2M NaOH and observed the formation of blue green color indicates the presence of anthocyanin.

### Statistical analysis

The experiment was conducted in triplicate across 2 separate sampling days The data acquired from each experiment was subjected to two-way ANOVA analysis, followed by Student's t-test considering a significance level of 0.01% and 0.05% respectively.

### Results and Discussion

The antimicrobial activity of *Bacopa monnieri* methanolic extract (BmMe) was evaluated by measuring the diameter of the zone of inhibition (ZOI) around each well. Remarkably, BmMe exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains.

The experiment was run in triplicates and the average diameter of zones of inhibition (ZOI) was reported for all the tested organisms interestingly the diameters increased progressively with higher concentrations of BmMe, indicating a concentration-dependent response. On Day 5 (Table 2), the antimicrobial efficacy of BmMe peaked,

surpassing the ZOI diameters observed on Day 1 (Table 1). This enhancement in efficacy suggests a potentiated effect over time.

For Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus mutans*, the ZOI diameters expanded dramatically from Day 1 to Day 5, indicating a substantial increase in antimicrobial activity.

*Staphylococcus aureus* (Figure 2) exhibited ZOI diameters ranging from  $7.40 \pm 0.1$  mm to  $12.70 \pm 0.8$  mm on Day 1 and  $12.27 \pm 0.2$  mm to  $23.47 \pm 0.5$  mm on Day 5, while *Streptococcus mutans* (Figure 3) showed ZOI diameters from  $11.53 \pm 0.4$  mm to  $20.53 \pm 0.1$  mm on Day 1 and  $13.47 \pm 0.1$  mm to  $23.10 \pm 0.4$  mm on Day-5. Similarly, Gram-negative bacteria also experienced significant growth in ZOI diameters from  $9.24 \pm 0.1$  mm to  $13.29 \pm 0.2$  mm on Day 1 and  $11.50 \pm 0.3$  mm to  $20.77 \pm 0.3$  mm on Day 5 for *Escherichia coli* (Figure 4) and from  $8.52 \pm 0.2$  mm to  $13.29 \pm 0.2$  on Day 1 and  $7.90 \pm 0.2$  mm to  $14.17 \pm 0.3$  mm Day 5 for *Pseudomonas aeruginosa* (Figure 5). This underscores the time-dependent enhancement in antimicrobial efficacy, indicating the extract's ability to exert prolonged inhibitory effects on bacterial growth. Phytochemical screening of the methanolic extract of *Bacopa monnieri* (Table 3) revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids. Notably, anthocyanin was found to be absent in the extract.

Embarking on an age-old journey, plants have stood as revered repositories of healing, offering remedies for a vast array of medical ailments, including those borne from microbial adversaries. Many modern drugs are derived from natural sources, with inspiration for novel therapies often originating from nature. Indigenous plant remedies have contributed significantly to the development of pharmaceuticals, with about 60% of the global population still relying on traditional medicine (Das et al., 2014). *Bacopa monnieri* contains compounds like flavonoids, tannins, and phenolic acids, which exhibit antimicrobial properties. Additionally, the plant has been studied for its pharmacological effects, including treating ulcers, inflammation, anemia, epilepsy, and asthma, as well as its antioxidant and anticancer potential (Mkk et al., 2019; Tamboli et al., 2022). The findings of the current investigation demonstrated that the methanolic extract derived from *Bacopa monnieri* exhibits antibacterial activity against pathogenic microorganisms affecting humans, encompassing both Gram positive and Gram negative strains.

**Table.1** Antimicrobial efficacy of *Bacopa monneri* methanolic extract (BmMe) against Gram positive (*S.aureus*, *S.mutans*) and Gram negative (*E.coli*, *P.aeruginosa*) reported in Average ZOI observed on Day 1 of the assay as the experiment was performed in triplicate for all organisms.

Concentration of BmMe (mg/ml)	Average Zone of Inhibition (ZOI) (mm) DAY 1			
	Gram positive		Gram negative	
	<i>S.aureus</i>	<i>S.mutans</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
1	7.40 ± 0.1	11.53 ± 0.4	9.24 ± 0.1	8.52 ± 0.2
2	8.27 ± 0.5	13.57 ± 0.6	11.12 ± 0.4	10.50 ± 0.4
3	8.40 ± 0.4	14.60 ± 0.2	11.26 ± 0.5	11.43 ± 0.6
4	9.31 ± 0.2	16.85 ± 0.4	12.38 ± 0.4	11.70 ± 0.4
5	11.43 ± 0.6	19.07 ± 0.3	12.56 ± 0.9	12.55 ± 0.3
6	12.70 ± 0.8	20.53 ± 0.1	13.43 ± 0.4	13.29 ± 0.2

**Table.2** Antimicrobial efficacy of *Bacopa monneri* methanolic extract (BmMe) against Gram positive (*S.aureus*, *S.mutans*) and Gram negative (*E.coli*, *P.aeruginosa*) reported in Average ZOI observed on Day 5 of the assay as the experiment was performed in triplicate for all organisms.

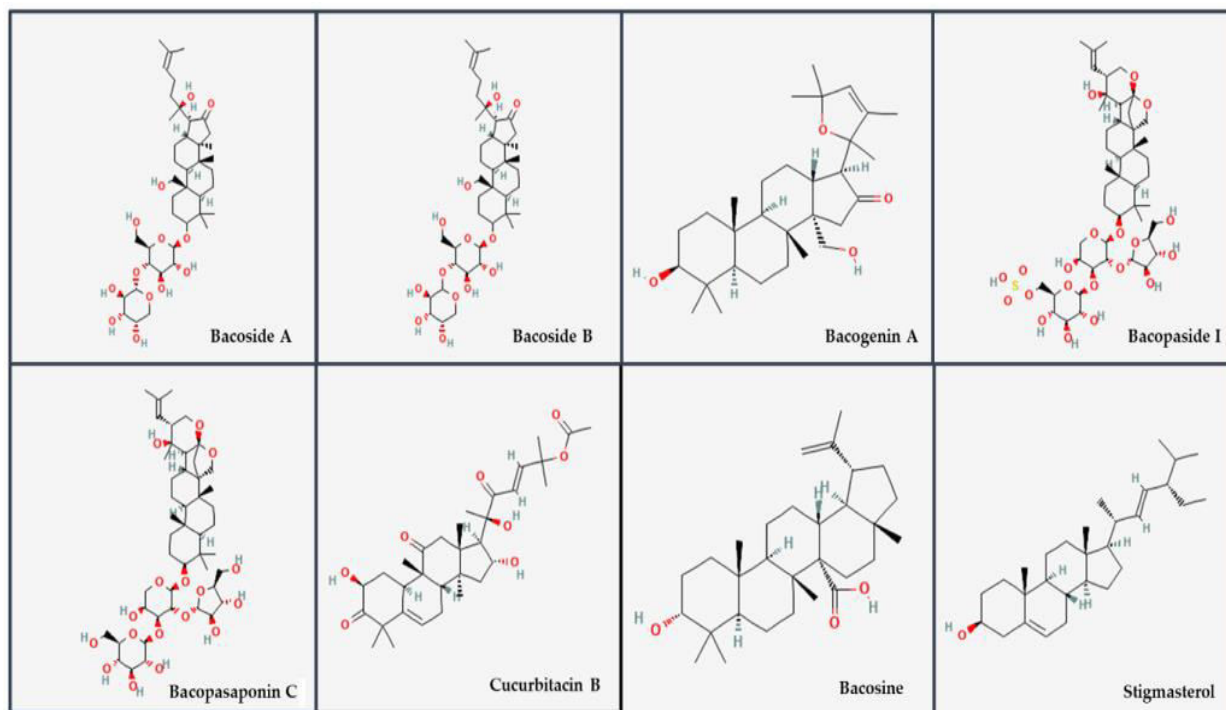
Concentration of BmMe (mg/ml)	Average Zone of Inhibition (ZOI) (mm) DAY 5			
	Gram positive		Gram negative	
	<i>S.aureus</i>	<i>S.mutans</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
1	12.27 ± 0.2	13.47 ± 0.1	11.50 ± 0.3	7.90 ± 0.2
2	15.13 ± 0.4	16.47 ± 0.6	14.27 ± 0.2	9.23 ± 0.5
3	17.33 ± 0.2	18.50 ± 0.2	16.17 ± 0.4	10.37 ± 0.4
4	19.73 ± 0.6	20.47 ± 0.5	17.73 ± 0.6	11.60 ± 0.9
5	21.63 ± 0.1	22.30 ± 0.3	19.33 ± 0.1	13.20 ± 0.6
6	23.47 ± 0.5	23.10 ± 0.4	20.77 ± 0.3	14.17 ± 0.3

**Table.3** Phytochemicals Analysis of methanolic extract of *Bacopa monneri* (Brahmi)

Sr. no.	Phytochemicals	Test	Results/observations
1	Alkaloids	Wagner’s test	++
2	Flavonoids	NaOH test	+++
3	Tannins	FeCl3 test	+++
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski’s test	+++
6	Steroids	Liebermann-burchard test	++
7	Anthocyanin	NaOH test	-

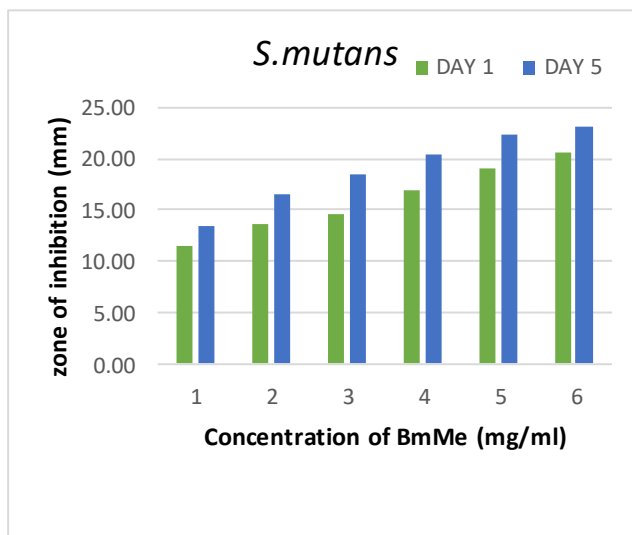
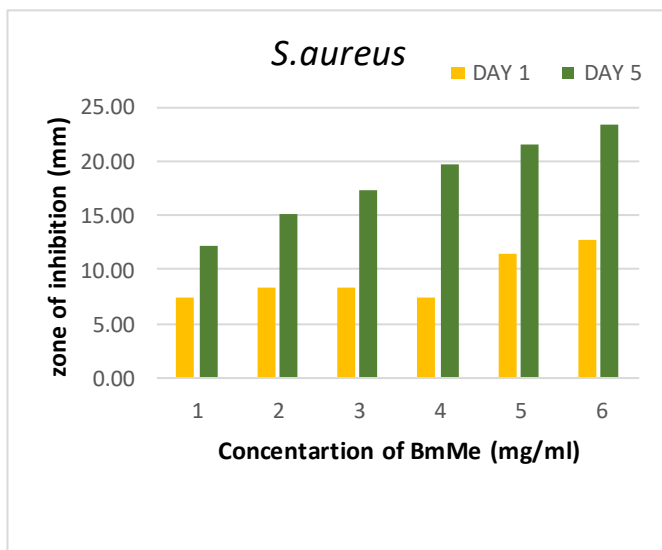
(+) indicates presence in trace concentrations  
 (++) indicates presence in moderate concentrations  
 (+++) indicates presence in high concentrations  
 (-) absent

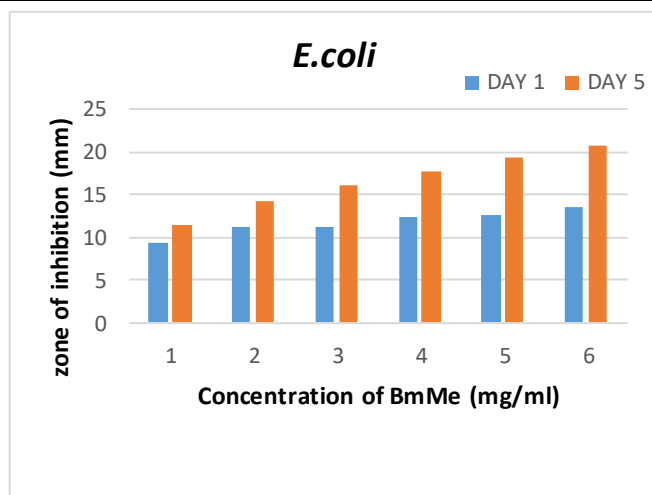
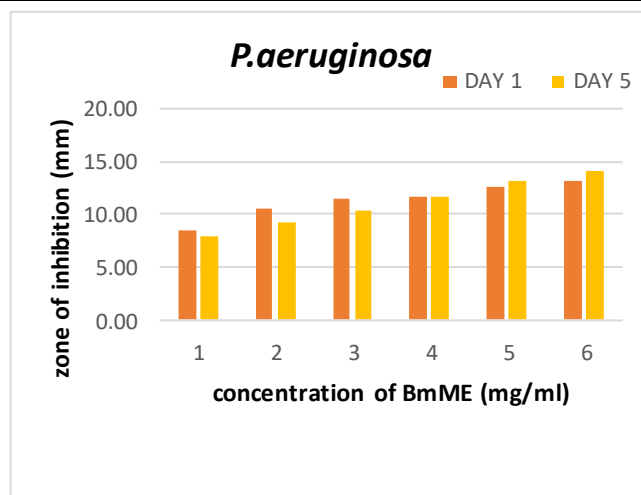
**Figure.1** Representative structures of major phytoconstituents present in extracts of *Bacopa monnieri*. Structures were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) with their corresponding PubChem CIDs, Bacoside A (92043183), Bacoside B (121596009), Bacogenin A (101600046), Bacopaside I (21599442), Bacopasaponin C (21599443), Cucurbitacin B (5281316), Bacosine (71312547), and Stigmasterol (5280794).



**Figure.2** Comparison of Average ZOI observed on Day 1 & 5 of assay against *S.aureus*.

**Figure.3** Comparison of Average ZOI observed on Day 1 & 5 of assay against *S.mutans*.



**Figure.4** Comparison of Average ZOI observed on Day 1 & 5 of assay against *E.coli*.**Figure.5** Comparison of Average ZOI observed on Day 1 & 5 of assay against *P.aeruginosa*.

This discovery corresponds with past research studies that have been documented in the literature (Khan *et al.*, 2010; Mehta *et al.*, 2022; Mkk *et al.*, 2019; Verma, 2014). The *Bacopa monnieri* methanolic extract (BmMe) demonstrated potency against all four tested organisms, albeit with varying degrees of effectiveness. Gram positive strains, including *S. aureus* and *S. mutans*, were the most inhibited compared to the Gram negative strains, *E. coli* and *P. aeruginosa* with moderate inhibitions.

This discrepancy may stem from the structural differences between Gram positive and Gram negative bacteria. Gram positive bacteria possess only an outer peptidoglycan layer, which serves as a less effective permeability barrier. In contrast, Gram negative bacteria have an outer phospholipidic membrane, along with structural lipopolysaccharide components, rendering their cell wall more impermeable to drug constituents (Rajashankarappa *et al.*, 2008). In addition to the differences observed between the two bacterial groups, the extract also exhibited notable variation when sampled after a 5-day interval, displaying significantly heightened antimicrobial activity compared to the initial day. This marked increase could be attributed to either a gradual release of bioactive compounds over time or due to the oxidation of phytochemicals, leading to intensified microbial inhibition.

Based on the comprehensive evaluation of *Bacopa monnieri* methanolic extract (BmMe), this study

confirms its potent antimicrobial activity against both Gram-positive and Gram-negative bacterial strains. Results indicate a concentration-dependent response in the zone of inhibition (ZOI) diameters, with a notable increase in antimicrobial efficacy on Day 5 compared to Day 1. This time-dependent enhancement underscores the extract's capacity to exert prolonged inhibitory effects on bacterial growth, potentially due to the gradual release of bioactive compounds or oxidation of phytochemicals.

Statistical evaluations, such as two-way ANOVA and Student's t-test, were performed to ascertain the significance of the findings. Both tests performed confirm the reliability of the experimental results. Phytochemical analysis of the methanolic extract of *Bacopa monnieri* identified alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids, with the absence of anthocyanin. These phytochemical constituents, known for their antimicrobial properties, contribute to the observed antibacterial activity of BmMe. Additionally, the traditional medicinal uses of *Bacopa monnieri* encompass the treatment of ulcers, inflammation, anemia, epilepsy, asthma, antioxidant, and anticancer properties. In summary, this investigation underscores *Bacopa monnieri* as a valuable natural reservoir with notable antimicrobial potential, substantiated by its phytochemical composition and pharmacological properties. Further exploration into its effectiveness in mouthwash formulations are warranted, potentially offering a fresh approach to oral hygiene maintenance.

## Future prospectus

*Bacopa monnieri*, hailed for its multifaceted medicinal attributes, emerges as a promising candidate for future utilization as an oral rinse. Acknowledged for its antiulcer, antioxidative, and potential anticarcinogenic properties, in addition to its functions as a relaxant, antidepressant, and cognitive booster, *Bacopa monnieri* demonstrates its adaptability as a botanical household remedy for humans (Tamboli *et al.*, 2022).

Its antioxidant properties make it particularly beneficial for neutralizing free radicals in the oral cavity, while its antiulcer effects may contribute to oral health maintenance.

Furthermore, its purported capacity as a cognitive enhancer and its forthcoming impact on the management of Alzheimer's disease could encompass beneficial roles (Dubey and Chinnathambi, 2019). Moreover, owing to its antioxidative characteristics, it may also discover applications in food supplements and enhance the health profile of food products (Shankar *et al.*, 2018). Through a blend of its historical usage and contemporary scientific discoveries, incorporating *Bacopa monnieri* into oral hygiene products can introduce a novel pathway for enhancing oral well-being. Additionally, exploring its potential as a natural disinfectant and its role in combating neurodegenerative disorders could pave the way for creating future prospects in healthcare and overall well-being for humanity.

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## Author Contributions

Mr. Anil kumar G Prajapati: Conceived the original idea and designed the model and wrote the manuscript; Mr. Bony Dasari: Designed the model and the computational framework, analyzed the data, supervised the overall project.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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