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Ophytochemical and Antibacterial Activity of *Bacopa monniera* Against the Common Bacterial Isolates from Human

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Abstract: Antibiotic resistance has become a global concern. Phytochemicals are the plant-derived naturally occurring compounds showed antimicrobial activity. and and *Bacopa monniera* has been used for centuries in the Ayurveda, a holistic system of medicine originating from India. In the present study phytochemical estimation (phenolics, flavonoids and carotenoids) and antibacterial activity of aqueous and ethanolic extracts of *B. monniera* was tested against the common bacterial isolates (*Bacillus subtilis, Escherichia coli, Klebsiella pnemonia, Pseudomonas auroginosa, Staphylococcus aureus, Streptococcus pyogenes, Shigella flexneri* and *Salmonella typhi*) from human by *Kirby-Bauer*'s disk diffusion and minimum inhibitory concentration (MIC) methods. The ethanolic extract of *B. monniera* has found to posses high phytochemical content than aqueous extract. In anti-bacterial activity, ethanolic extract showed high inhibitory action against the tested bacterial strains by both disk diffusion and MIC methods than aqueous extract. In conclusion, both aqueous and ethanolic extracts of *B. monniera* have antibacterial activity against the bacterial strains tested.

Key words: Phytochemicals · *Bacopa monniera* · Kirby-Bauer's disk diffusion · Minimum inhibitory concentration

INTRODUCTION

Over the past years the problem of antimicrobial resistance received increasing attention and it becomes a global concern [1]. In addition to the increased magnitude of emergence of bacterial drug resistance, high-dosage and prolonged antimicrobial therapy could eliminate the commensal beneficial bacterial and predisposing to pathogen invasion [2, 3]. There is, thus isolation of microbial agents less susceptible to regular antibiotics and rising trend in the recovery rates of resistant bacteria which highlights the need for newer alternative principles. There has been a worldwide move towards the use of traditional medicines due to the concerns over the more invasive, expensive and potentially toxic mainstream practices. Traditional healing systems around the world that utilize herbal remedies are an important resource for the discovery of new antibiotics. Studies have identified compounds within herbals that are effective antibiotics [4].

B. monniera belongs to the family Scrophulariaceae, commonly called as Brahmi, Neer brahmi etc. *B. monniera* is a small creeping herb with numerous branches, small oblong leaves and light purple or small and white flowers, with four or five petals. It is found in wetlands throughout the Indian subcontinent in damp and marshy or sandy areas near streams in tropical regions. *B. monneri* is reported for its tranquilizing [5], sedative [6] and antioxidant properties [7].

The present study investigates the phytochemical and antibacterial effect of aqueous and ethanolic leaf extracts of *B. monneri* against common bacterial isolates from human using the conventional *Kirby-Baue*r's disk diffusion and MIC methods.

MATERIALS AND METHODS

Plant Material: B. monniera was collected from the Anna Sidha College, Chennai, Tamil Nadu, India and taxonomically identified by, the Department of Botany,

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University of Madras, Chennai, India and specimens were deposited at an herbarium (No: CASBH12).

Aqueous Extract: The entire plant of *B. monniera* was grounded into fine powder, maintained at 60°C for 3 hr in sterile distilled water. The resulting suspensions were filtered and evaporated for dryness at 60°C *in vacuo*.

Ethanolic Extract: B. monniera (coarse powder) *was* put in a soxhlet extractor containing 70% of ethanol. The resulting extract was preserved at 5°C in an airtight bottle until further use.

PHYTOCHEMICAL STUDY

The phytochemical study deal's with the estimation of phenolics, flavonoids and carotenoids, present in the aqueous and ethanolic extracts of *B. monniera.*

Estimation of Phenolics: Isolation and estimation of phenolics was performed according the protocols suggested by Price *et al.* [8]. Briefly, 5g of sample was homogenized thoroughly in 200ml of acetone using mortar and pestle, transferred to a stoppered flask and kept overnight in a shaker. The supernatant was collected and the residue was extracted twice with 10 ml acetone. The collected extracts were pooled and filtered through Whatman No.1 filter paper and the filtrate was centrifuged at 3000 x g for 10 minutes. The resultant supernatant was used for the estimation of total phenolics. One milliliter of distilled water was added to 25μl of extract. To this 25μl of acetone and 60μl of ferric ammonium sulphate were added and kept at room temperature for 20 minutes. Then 60μl of potassium ferricyanide was added and the absorbance was measured at 720 nm after 20 minutes using quercetin as standard. The total phenolic was expressed as μg/gram of extract.

Isolation and Estimation of Flavonoids: The isolation and estimation of flavonoids was carried out using protocols of Harborne [9] and Lamaison and Carnat [10]. Briefly, 5g of sample was acid hydrolysed with 10ml of 1N Sulphuric acid at 70°C for 1 hour and neutralized with 0.5ml of 10N sodium hydroxide. To this 5ml of ethyl acetate was added, shaken well and the ethyl acetate portion was collected. This was repeated thrice and the ethyl acetate was pooled and evaporated to dryness. The residue was reconstituted with 1ml of HPLC grade methanol and assayed for total flavonoid content. From the extract, 1 ml of extract was mixed with 1ml of 2% methanolic $AlCl_3$ and the absorbance was measured at 430 nm.

Isolation and Estimation of Carotenoids: The isolation and estimation of carotenoids was performed by protocols suggested by Naryanaswamy and Palanisamy [11]. Briefly, 5g of sample was homogenized in 20ml of acetone using mortar and pestle and filtered using Whatman No. 1 filter paper. The extraction was repeated until it was free from pigments. The filtrates were pooled and partitioned with equal quantity of peroxide free ether, thrice using the separating funnel. The ether phase containing the carotenoids was evaporated and the residue was dissolved in 1ml ethanol. Then 0.1ml of 60% KOH was added and partitioned twice into peroxide free ether. The ether was evaporated and the residue was dissolved in 0.5ml ethanol and used for measurement of carotenoids. The extract was dissolved and its absorbance was measured at 450nm

Disk Preparation: Sterile blank diffusion disks were placed into labelled trays for each ethanol or aqueous extract. A 10% of extract concentration was prepared with the respective solvents and from this preparation, disks were prepared by saturation with 5μl of the individual aqueous or ethanol extracts. Control disks were prepared by saturating sterile blank disks with ethanol and sterile distilled water and allowing the solvent to evaporate.

Bacterial Dilution: The bacterial cultures were identified by standard culture and biochemical methods in the microbiology laboratory. The organisms were maintained on agar slope at 4°C and sub-cultured for 24 hr before use. Isolated colonies of the bacteria were placed into individual tubes containing 5 ml of sterile brain-heart infusion broth (BHIB) (Himedia, Mumbai, India) and incubated at 37°C, before adjusting the tubes with 0.5 McFarland Units using sterile BHIB. Turbidity was also verified using spectrophotometrical comparison with a 0.5 McFarland Standard. The dilutions were used within 15 minutes of their preparation and were vortexed prior to use.

Disk Diffusion Analysis: The standardized inoculum ($1\text{-}2 \times 10^7$ cfu/ml 0.5 McFarland standards) was introduced on the surface of sterile Mueller-Hinton agar (Himedia, Mumbai, India) (MHA) (pH 7.2 to 7.4) plates using sterile swab. The inoculations were done along three axes in a rolling motion to ensure uniform bacterial distribution and growth. After the plates were labelled, the disks were placed on the surface of the agar. The plates were inverted and incubated at 37° C for 16 hrs. Measurements were made from the (average mean of triplicates) outer edge of the disks to the inner edge of any zones of clearance produced.

Minimum Inhibitory Concentration (Mic): The minimum inhibitory concentration (MIC) values were determined using MH broth-dilution method. The aqueous and ethanolic extracts were sterilized by 0.45-mm Millipore filters and added to MH broth medium. Serial 10-fold dilutions were made that furnished a concentration range from 0.01 to 10,000 mg/ml for both aqueous and ethanolic extracts. The tubes were incubated aerobically at 37°C for 18-24 hrs. Two control tubes were maintained. These include antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

RESULTS

Yield: The aqueous and ethanolic extracts yield of *B. monniera* was 9 and 14%, respectively (Table. 1).

Phytochemical Content: The results of various phytochemicals content present in *B. monniera* extracts are given in Table 2. The estimation of phytochemical content has revealed that one gram of crude aqueous extract of *B. monniera* contains 6620µg of phenolic content, 612 µg of flavonoids and 240µg of carotenoids, while ethanolic extract had 96500µg of phenolic content, 610µg of flavonoids and 422µg.

Disk Diffusion Analysis: No zones of clearance were produced by the solvent-only control disks (Table 3). The aqueous extract of *B. monniera* produced zone of clearance measuring less than 4mm against the bacterial strains studied. Ethanolic extracts of *B. monniera* showed consistence zone of clearance measuring greater than 3mm against *Escherichia coli, Pseudomonas auroginosa, Staphylococcus aureus, Streptococcus pyogenes, Shigella flexneri* and *Salmonella typhi* and less than 3mm against *B. Substilis* and K. pneumoniae.

Table 1: The aqueous and ethanolic extracts yield of *B. monniera.*

Plant	Extract	Yield in %
B. monniera	Aqueous	9
B. monniera	ethanol	14

Table 2: The phytochemicals namely Phenolics, Flavonoids and Carotenids, in aqueous and ethanol extracts of *B. monniera*

B. monniera	Phenolics (µg/g)	Flavonoids (µg/g)	Carotenoids (µg/g)
Aqueous	6620	612	240
Ethanol	96500	610	422

Table 3: Antibacterial activity of aqueous and ethanolic extracts of *B. monniera* by Kirby-Bauer's disk diffusion

	Concentration (5 µl)	
Bacterial isolate	Aqueous	Ethanolic
Bacillus subtilis	+	+
Escherichia coli	+	++
Klebsiella pnemonia	+	+
Pseudomonas auroginosa and	+	++
Staphylococcus aureus	+	++
Streptococcus pyogenes	+	++
Shigella flexneri	+	++
Salmonella typhi	+	++
Solvent (aqueous/ethanol)	-	-

'-' no antibacterial activity, '+' Zone of inhibition less than 3 mm, '++' zone of inhibition 3 - 6 mm

MIC: In MIC, *Escherichia coli, Pseudomonas auroginosa, Staphylococcus aureus, Streptococcus pyogenes, Shigella flexneri* and *Salmonella typhi* were sensitive at low concentrations of aqueous (100µg/ml) and ethanolic (10µg/ml) extracts. The bacterial strain *B. substilis* and *K. pneumoniae* were sensitive at 1000µg/ml of aqueous and 100µg/ml ethanolic extracts, respectively.

DISCUSSION

The present study investigated the antibacterial effect of aqueous and ethanolic extracts of *B. monniera* against common bacterial strains from human. It has been suggested that ethanolic and aqueous extracts from plants are potential sources of antimicrobial agents [12]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [13]. Hence in the present study aqueous and ethanolic crude extracts of *B. monniera* was studied. Both aqueous and ethanolic leaf extracts of *B. monniera* were found to posses inhibitory effects against the bacterial strains tested in both disc diffusion (Table 3) and MIC methods (Table 4). *B. monniera* showed antibacterial effect on both Gram-positive as well as Gram-negative bacteria suggests the passage of active phytochemicals through both the bacterial cell walls.

Table 4: Antibacterial activity of aqueous and ethanolic extracts of *B. monniera* by Minimum inhibitory concentration method (μg / ml).

Bacterial isolate	Concentration (5 μl) Aqueous	Ethanolic
Bacillus subtilis	1000	100
Escherichia coli	100	10
Klebsiella pnemonia	1000	100
Pseudomonas auroginosa and	100	10
Staphylococcus aureus	100	10
Streptococcus pyogenes	100	10
Shigella flexneri	100	10
Salmonella typhi	100	10

Minimum inhibitory concentration values are expressed as μg/ml

Phytochemicals like phenolics, flavonoids and carotenoids have been reported to have antibacterial activities [14, 15]. In the present study ethanolic extracts showed high inhibitory action against the studied bacterial strains than aqueous extracts. This might be due to the lesser solubility of the active constituents in aqueous solutions, resulting in less antibacterial effect. The ethanolic extract of *B. monniera* yield was more than the aqueous extract. Addition to that, phytochemical estimation showed the existence of higher phytochemical content in ethanolic extract of *B. monniera* (Table 2). Since most of the antibiotic compounds already identified in herbs are reported as aromatic or saturated organic molecules, making ethanol an ideal solvent [16].

Nevertheless, *B. monniera* reported to contain numerous phytochemicals including, the existence of other ingredients warrants more studies. Moreover, the mechanisms behind the antibacterial activity are complex to understand and could be attributed to either inhibiting the cell division or damaging the cell walls of bacteria, which however requires to be further investigated in detail. In conclusion, this study showed *B. monniera* has potent antibacterial action against the studied bacterial strains. This antibacterial activity may be due to the presence of phytochemicals, which warrants *B. monniera* be subjected to extensive preclinical and clinical antibacterial experimentation in the future to treat certain disease caused by the studied bacterial strains.

It was concluded that *Bacopa monniera* is likely a potential medicinal plant resource for developing effective antibacterial.

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