Antibacterial Activity of Bacoside-A- an Active Constituent Isolated of *Bacopa Monnieri* (L.) Wettest.

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Summary

The antibacterial activity of the crude methanolic extract and the isolated constituent bacoside-A of *Bacopa monnieri* were screened against 30 clinical strains isolated from different infectious sources which belonging to gram-negative *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae,* and gram-positive *Staphylococcus aureus*.

The minimal inhibitory concentrations of the methanolic extract and the constituent bacoside-A were determined against American Type Cell Culture (ATCC) and Microbial Type Cell Culture (MTCC, India) strains. Concentrations higher than 100 µg/100 µl of the methanolic extract and 50 µg/100 µl of the constituent bacoside-A indicated that their effect was bacteriostatic.

The agar wells loaded with bacoside-A and the methanolic extract exhibited a significant zone of inhibition against the clinical strains of *S. aureus* (21.33±0.33mm) isolated form the puss of wound samples of infected patients. A moderate zone of inhibition was observed on the clinical strains of *K. pneumonia* (16.18±0.13mm) and *P. aeruginosa* (14.08±0.16mm).

The antibacterial activity of bacoside-A was promising against gram positive *s. Aureus*, comparative with the standard drug ciprofloxacin (50 µg/100 µl).

Kew Words: *Bacopa monnieri,* Antibacterial activity, Clinical isolates, *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa*

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Introduction

Bacopa monnieri (L.) Wettest. (Scrophulariaceae), is a well-known medicinal herb in Indian system of medicine as Brahmi (Sanskrit) and Indian water hyssop. The plant is commonly found in wet, damp and marshy areas. Indian Materia Medica (1500 AD) cites the uses of this plant as a brain tonic, which is effective in maintaining the vigor and intellect (Anonymous 1998)**.** Compound, which is, responsible for the memory enhancing is a triterpenoid saponin called 'Bacosides'. Bacosides enhance the efficiency of transmission of nerve impulse there by strengthening memory and cognition[1]. It is also used as a laxative and curative for ulcers, inflammation, anemia, scabies, leucoderma, epilepsy and asthma[2]. The plant is also reported to show sedative [3], hyperthyroidism[4], vasoconstrictor, anti-inflammatory[5] and gastrointestinal disorder [6].

Antimicrobial resistance among key microbial pathogens continues to grow at an alarming rate[7,8]. In many parts of the world fluoroquinolone antibiotics (pefloxacin, ciprofloxacin and ofloxacin) are recommended for serious infections associated with *Klebsiella, Pseudomonas* and *Staphylococcus* species. The increased prevalence of antibiotic-resistance bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some bacterial diseases[9]. Plants produce highly bioactive molecules that allow them to interact with other organisms in their environment. These bioactive compounds are important in defense mechanism and contribute to the resistance to diseases. Many investigators evaluated the bioactivity of plant extracts and the constituents against the serious infectious organisms[10,11].

In the present investigation the antibacterial activity of the methanolic extract and Bacoside-A were screened against pathogenic clinical strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from different infectious sources of the patients.

Materials And Methods

Plant Material

The cultivar 'Pragyashakthi' used for extraction from the Medicinal garden, Division of Horticulture, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore, and

Karnataka, India. The material was air dried in shade, powdered mechanically and stored in airtight containers. One kg of the powdered material was refluxed with methanol (800 mL) in a soxhlet apparatus for 48 h in batches of 250 g each. The extract was filtered, pooled and the solvent was removed under reduced pressure at 40 ± 5^0 C using rotary flash evaporator (Buchi, Flawil, Switzerland).

Isolation

The constituent bacoside-A was isolated from the methanolic extract of the *B. monnieri.* The crude methanolic extract was taken and dissolved in n-butanol, then n-butanol solubles were again solubilized in ethyl acetate, ethyl acetate insoluble residue was taken and chromatographed on silica gel column using toluene, ethyl acetate, methanol and glacial acetic acid in the ratio of 3:4:3:1. The eluted fractions were collected at an interval of 5 min and they were divided into five fractions, each fraction was monitored by thin layer chromatography to check the purity of the compound. Then the compound was dried to remove the solvent by evaporation. Based on the purity. These fractions were pooled and recrystallized. The crystalline compound was tested qualitatively for triterpene and saponins and the chemical identity of the compound was confirmed by IR (Shimadzu 8201 PC), 1H NMR (Bruker DRX-300), C13 NMR (Bruker Advance 400) and MASS (Micromass QuattroII) spectral studies.

Antibacterial Activity

The antibacterial activity of the methanolic extract and Bacoside-A were screened by agar-well diffusion method[12] against thirty clinical isolates of each of ten bacterial strains belonging to *Klebsiella pneumoniae, Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive) respectively. The bacterial strains used for screening of antimicrobial activity were collected from different infectious status of the patients who had not taken any antibacterial drugs for at least two weeks with the help of authorized physician, in District Health Center of Gulberga, Karnataka state, India. The clinical isolates were identified following the standard method[13]. The bacterial suspensions were diluted at 10^{-1} to 10^{-8} phosphate buffered saline. Samples were homogenized and then loaded in six amounts of 20µL on to Nutrient agar plates. The plates were incubated for 24 h at 37^0 C and counting was done on plates containing 50 to 100 colonies.

The comparative activity was screened with the reference to ATCC strains (*Pseudomonas aeruginosa*- ATCC-20852; *Staphylococcus aureus*- ATCC 29737) and MTCC strain (*Klebsiella pneumoniae* – MTCC-618). The fluoroquinolone antibiotic Ciprofloxacin was used as the standard (50 µg /100µL of sterilized distilled water) concomitantly with the test samples.

The minimal inhibitory concentrations (MIC) of the crude methanolic extract and Bacoside-A were determined by micro dilution techniques in nutrient broth, following National Committee for Clinical Laboratory Standard, USA guidelines. The inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The micro titer plates were incubated at 37^0 C and MIC was determined after 24 h of incubation.

A sensitive radial diffusion technique was used for the assessment of antibacterial activity of the test samples. Sterilized Nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 100µL of 24 h incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a sterilized cork borer in an aseptic condition. 100mg of dried methanolic extract and 50mg of powdered Bacoside-A was dissolved in 10 mL of 10% DMSO. 100µg /100µL of the methanolic extract and 50µg /100µL of Bacoside-A were loaded in the corresponding wells. The standard drug Ciprofloxacin was tested at the concentration 50µg/100µL. The plates were incubated for 24 h at 37^0 C and the diameter of the zone of complete inhibition of the bacteria was measured around each well and readings were recorded in mm.

Statistical Analysis

The data obtained from each of experiment were subjected to one-way ANOVA followed by Tukey's pairwise comparison Test. In analysis of variance (ANOVA) the calculated F-value is compared with the standard table F-value for particular degree of freedom at 0.05% level of significance. If calculated F-value is more than tabulated F-value than it is significant at 0.05% level of significance otherwise it is not significant.

Results

The isolation of authentic samples for all the detectable triterpenoid saponins in *B. monnieri* extract has given us the opportunity to develop an analytical procedure to study the chemical composition of Bacoside-A. The white crystalline eluted from the methanolic extract of Bacopa monnieri showed positive test for terpenes, saponins. The chemical structure of the compound was confirmed by IR, 1H NMR, C13 NMR and MASS spectral analysis[14].

The ten different clinical strains of *Staphylococcus aureus* were isolated form the puss samples of the old wounds of the infected patients. The isolated constituent Bacoside-A was most effective in controlling the growth of these clinical strains and also the ATCC strain (Table 1) compare with methanolic extract. The significant zone of inhibition was observed in the wells loaded with the Bacoside-A (21.33±0.33) which is nearer to the value of standard antibiotic Ciprofloxin (21.57±0.07). Many researchers attempted to verify the synergism between antimicrobial drugs and plant extracts against the pathogenic *Staphylococcus* aureus[15]. In the present investigation the Bacoside-A of *Bacopa monnieri* was most effective in controlling the growth of the pathogenic strains of *Staphylococcus aureus* isolated directly from the wounds of the patients.

Among different clinical strains the gram negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from the urine samples of urinary tract infected patients, the Bacoside-A and methanolic extract loaded wells were showed moderate zone of inhibition when compared to Ciprofloxin loaded well. The zone of inhibition of the colony of ATCC strain of *Pseudomonas aeruginosa* and MTCC strain *Klebsiella pneumoniae* was similar to the culture of clinical isolates and the data is depicted in the (Table 2 and Table 3).

Table 1: Antibacterial activity of Methanolic extracts and Bacoside-A aganist *Staphylococcus aureus*

Bacterial strains tested		**Zone of inhibition (in mm)**		
Staphylococcus aureus	**Source of collection**	**Methanolic extract**	**Bacoside-A**	**Reference drug Ciproflaxin**
	ATCC-29737	21.23±0.49	21.33±0.33	21.57±0.07
Sa-1	Wounds	21.13±0.58	21.18±0.05	19.00±0.26
Sa-2	Wounds	22.00±0.58	21.25±0.03	20.67±0.26
Sa-3	Pus	21.42±0.64	21.08±0.13	21.00±0.23
Sa-4	Urine	19.63±0.19	20.85±0.04	21.34±0.20
Sa-5	Mucus	20.33±0.33	20.97±0.07	21.20±0.26
Sa-6	Urine	21.00±0.21	20.32±0.13	21.10±0.53
Sa-7	Burn wounds	21.33±0.18	20.97±0.13	21.17±0.22
Sa-8	Urine	20.55±0.17	20.60±0.05	21.33±0.17
Sa-9	Hospital effluents	19.33±0.20	20.95±0.08	22.85±0.15
F-Value		27.1	14.2	10.0

The value of each constituents consisted of ± S.D. of 06 replicates.

The F value is significantly different when $p < 0.05\%$.

Table 2: Antibacterial activity of Methanolic extracts and Bacoside-A against *Klebsiella pneumoniae.*

Bacterial strains tested		**Zone of inhibition (in mm)**		
Klebsiella pneumoniae	**Source of collection**	**Methanolic extract**	**Bacoside-A**	**Reference drug Ciproflaxin**
	MTCC-618	15.00±0.58	16.18±0.13	20.17±0.67
Kp-1	Urine	13.00±0.58	15.98±0.12	17.33±0.33
Kp-2	Urine	14.00±0.58	15.17±0.09	17.00±0.58
Kp-3	Urine	13.33±0.33	14.75±0.11	17.67±0.67
Kp-4	Urine	13.40±0.33	14.23±0.12	17.67±0.33
Kp-5	Urine	13.67±0.38	14.98±0.12	17.33±0.67
Kp-6	Urine	13.33±0.88	14.50±0.20	17.33±0.33
Kp-7	Urine	13.67±0.33	14.58±0.10	18.00±0.58
Kp-8	Urine	13.00±0.58	14.68±0.18	19.45±0.58
Kp-9	Urine	14.67±0.88	14.73±0.22	20.63±0.64
F-Value		13.5	17.5	5.2

The value of each constituents consisted of ± S.D. of 06 replicates.
The F value is significantly different when $p < 0.05\%$.

Table 3: Antibacterial activity of Methanolic extracts and Bacoside-A against *Pseudomonas aeruginosa*

Bacterial strains tested	Source of collection	Zone of inhibition (in mm)		
Pseudomonas aeruginosa		**Methanolic extract**	**Bacoside-A**	**Reference drug Ciproflaxin**
	ATCC -20852	13.33±0.95	14.08±0.16	16.77±0.33
Pa-1	Urine	12.30±0.31	13.67±0.13	17.33±0.33
Pa-2	Urine	13.00±0.37	13.08±0.22	18.00±0.58
Pa-3	Ear swab	13.33±0.24	13.58±0.16	18.00±0.58
Pa-4	Pus	13.33±0.67	13.12±0.09	17.33±0.33
Pa-5	Urine	11.67±0.87	13.17±0.21	20.58±0.40
Pa-6	Urine	11.68±0.23	13.37±0.12	18.67±0.48
Pa-7	Stool	12.33±0.33	13.37±0.12	17.0±0.58
Pa-8	Hospital effluent	12.67±0.33	13.68±0.16	18.00±0.58
Pa-9	Sputum	13.00±0.58	13.62±0.17	18.67±0.88
F-Value		35.4	3.52	13.6

The value of each constituents consisted of ± S.D. of 06 replicates.
The F value is significantly different when $p < 0.05\%$.

Discussion

The clinical strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant to multi drugs and they were highly disruptive to the internal epithelial barrier and cause lethal sepsis with in the intestinal tract[16]. Generally the gram-positive bacteria should be more susceptible having only an outer peptidoglycon layer which is not an effective permeability barrier[17]. Where as, the gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents. Most clinical isolates displayed multiple antibiotic resistances to various antibiotics clinically used against both gram-positive and gram-negative strains. In the present study the methanol extract of *Bacopa monnieri* exhibited more significant inhibitory effect on gram positive strains of *Staphylococcus aureus.* The gram-positive *Staphylococcus aureus* causes a variety of supperative (pus forming) infections and toxinoses in humans. It also causes superficial skin lesions such as boils and also more serious infections such as pneumonia, mastitis, phlebitis and meningitis. Reports indicated that clinical isolates from different infectious sources from hospitals showed resistance against the drug Methicillin [18,19]. The growth of inhibition was moderate in the cultures of gram negative strains of Pseudomonas *aeruginosa* and *Klebsiella pneumoniae* which were commonly infects the urinary tracts.

Medicinal plants are important elements of traditional medicine in virtually all cultures. The idea that certain plants had healing potential was known long before human being discovered the existence of pathogens. Medicinal plants which have been used by human being to treat common infectious diseases are important elements of traditional medicine. During the last years, traditional medicine has not been limited to specific culture. It has been used in developing countries as well as its using extended to developed countries [20]. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases. Single and Poly herbal preparations have been used numerously through out history for the treatment of various diseases. Many studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity[21].

In vitro studies on plants used in traditional medicine have been carried out in the field of microbiology, especially on pathogenic bacterial growth; and some of these studies were

about the antimicrobial activity of *Mikania glomerata* Spreng ("guaco")[22], *Psidium guajava* L (guava) [23,24] , *Syzygium aromaticum* (L) Merrill & Perry (clove) [25].

In most of the countries popular herbal medicines were used as remedies for many infectious diseases [26,27]. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics this has led many researchers to investigate the antimicrobial activity of medicinal plants.

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