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Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata P*. Beauv

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ABSTRACT

The ethanol extract of leaf and flower of *Spathodea campanulata* was investigated for antimicrobial activity at 10 mg/ml concentrations by using Kirby-Bauer disc diffusion method against gram positive and gram negative organisms like *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas sps, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera*. After incubation for 24 hrs, the zone of inhibition was compared with standard antibiotics genatmycin and streptomycin (10 µg/ disc). From the dose dependent study it was observed that the ethanol flower extract was more potentent than leaf extract. Flavonoids and tannins present in the both ethanol extract may be responsible for the antimicrobial activity.

Keywords: *Spathodea Campanulata,* antimicrobial activity, disc diffusion method, minimum inhibitory concentration (MIC)

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INTRODUCTION

Diseases caused by bacteria are widespread worldwide. The treatment of these infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes [1]. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions [2]. Therefore, there is a need to develop alternative antibacterial drugs for the treatment of infectious diseases from various sources such as medicinal plants.

Undoubtedly, medicinal plants are the prime source of drugs in both developing and developed nations, as drugs or herbal extracts for various chemotherapeutic purposes. There are about 2000+ plant species known to possess medicinal value in the traditional Asian system of medicine[3].The use of plant derived natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [4,5]. They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids, and essential oils. Over 50% of all modern clinical drugs are of natural origin [6] and play an important role in development of drugs [7,8]. Many herbs have been used for treating disease caused by microorganisms such as cholera, diarrhea, dysentery, Typhoid and bacterial enteritis [9]. Moreover, Huge economy is invested in the imports of drugs especially antibiotics from different parts of the world. Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative antibacterial regimens. In the continuation of this strategy of new drug discovery we have studied only the aerial parts of the plant *S. campanulata* for their antibacterial, cytotoxic and antioxidant properties.

Spathodea campanulata P. Beauv is a flowering plant belonging to the Bignoniaceae family. It is commonly known as the Fountain Tree, African tulip tree, Flame-of-the-forest, Rudra Palash, Pichkari or Nandi Flam [10]. It is a tree that grows between 7–25 m (23–82 ft) tall and is native to tropical Africa. Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, flowers, leaves, and fruits. Spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid and pectic substances have ever been isolated from the stem bark [11-14]. Flowers and stem bark extracts have shown molluscicidal activity. These are also employed in diuretic and anti-inflammatory treatments. Banerjee and DE [15] showed the presence of anthocyanins in flowers of *S. campanulata*. The stem bark preparations are used to treat fungus skin diseases, herpes, stomach aches and diarrhea [16]. Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts [17,18]. The leaves are used against kidney diseases, urethral inflammations and as an antidote against animal poisons. *In vitro* antimalarial activity against *Plasmodium falciparum* and antibacterial activity of bovine mastitis causing *S.aureus* were evaluated using leaf extracts of *S.campanulata* [19]. The leaves have been found to contain spathodol, caffeic acid, other phenolic acids and flavonoids [20-23]. *In vitro* antibacterial activity of leaf extracts of this plant

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against standard strains was evaluated [24]. The phenolic derivatives produced in *S. campanulata* roots fungitoxic properties that in vitro [25].

MATERIALS AND METHODS

Plant collection & extraction

Fresh plant leaves and flowers were collected from B.G. Nagar, Mandya (District), Karnataka, India.. Fresh plant material were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight containers. Same procedure was followed for both leaf and flower extraction. For Ethanol extraction, 100 g of air dried powder was extracted with ethanol (40-60^{o}C) in a Soxhlet extractor for 18-20 hr and solution was evaporated to dryness under reduced pressure and controlled temperature by using roto evaporator. The extract was stored in a refrigerator at 4 °C in air-tight bottles until further use. 10 mg/ml concentration of both leaf and flower was prepared in ethanol and used.

Phytochemical screening [26]

Phytochemical studies of ethanol extracts of *S. campanulata* flowers & leaves using standard procedures to identify the phytoconstituents (Table-3).

Bacterial culture

Authentic Clinical isolated pure culture of human pathogenic bacteria*, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas sps, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera* were obtained from Microbiology Department, Adichunchanagiri Institute of Medical Science (AIMS), B.G. Nagar, Karnataka, India. All the strains were confirmed by cultural and biochemical characteristics and maintained in slants for further use.

Evaluation of Antimicrobial Activity

Antimicrobial activity of each plant extract and was determined using a modified Kirby-Bauer [27,28]. disc diffusion method. Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria, 100 µl of microbial suspension was spread onto the Nutrient agar plates.

The extracts were tested using 5 mm sterilized filter paper discs. Discs were impregnated with 25 µl (10mg/mL concentration) of the test samples (ethanol leaf and flower extract), allowed to dry and placed onto inoculated plates (30 min incubation). The plates were allowed to stand at 4 ^{0}C for 2 hours before incubation with the test microbial agents. Plates inoculated with *E. coli, K. pneumonia, P. vulgaris, Pseudomonas sps, S. typhimurium, B. subtilis, S. aureus* and *V. cholera* were incubated at 37 ^{0}C for 24 hours, than the diameters of the inhibition zones were measured in millimetres. Each antimicrobial assay was performed in

triplicate & mean values were reported. Standard antibiotics, gentamycin (10 µg/ disc), streptomycin (10 µg/disc) served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as a negative control. Solvent control disc (ethanol) was also placed with the test, positive and negative control.

Dose dependent antibacterial activity

Both the leaf and flower ethanol extract was checked for the dose dependent antibacterial activity. Different concentration of extract (100, 200, 400, 600, 800 1000 µg/disc) was impregnated on to the disc and the same procedure was followed as mentioned before. Each assay was performed in at triplicate and mean of all the three experiment were taken.

Minimum inhibitory concentration

The MIC was done by the method described by Ver-poorte [29]. The extracts were incorporated into Mueller-Hinton broth at concentration ranging from 0.01-10mg/ml. A control tube containing the growth medium and the bacteria was set-up. The mixtures were incubated at appropriate temperature of 37°C for 24h. The minimum inhibitory concentration (MIC) of the extracts was regarded as the lowest concentration of the extract that did not permit and turbidity or growth of the test organisms.

RESULTS AND DISCUSSION

The antimicrobial activity of ethanol extract of leaves and flowers *S. campanulata* against human pathogenic bacteria*, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas sps, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera* were measured by measuring the zone of inhibition in disc diffusion method. Test sample per disc was about 250 µg/disc. The organisms used and zone of inhibition to the corresponding extracts are shown in Table 1. The Zone of inhibition ranged from 6 – 8 mm and 8.6 – 11.2 mm for leaf and flower extract respectively.

Antibacterial activity at different doses was done by disc diffusion method. Concentration was in the range of 100 to 1000 µg/disc. Activity was dependent on the dose of the test material. As the concentration increased the inhibition zone was also increased. Against ethanol leaf extract, *Proteus vulgaris, Escherichia coli* and *Klebsiella pneumonia* showed a highest inhibition zone of 16, 15 and 14 respectively where as *Staphylococcus aureus* showed a lesser inhibition zone of about 10 mm (Figure 1). Ethanol Flower extract showed more potency than the leaf extract. Against *Klebsiella pneumonia, Vibrio cholera, Staphylococcus aureus* and *Proteus vulgaris* it showed 19, 18.5, 18 and17mm at 1000 µg/disc. *Salmonella typhimurium* showed a lesser inhibition zone of 14 mm (Figure 2).

MIC value for both Leaf and flower extract against the bacterial strains were done by serial dilution method. MIC values for leaf extract ranged from 221 – 254 µg/mL and 156 – 173 µg/mL for leaf and Flower ethanol extract respectively. Against leaf extract, *E. coli* showed a

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minimum value of 221 µg/mL where as against flower extract, *P. vulgaris showed 156* µg/mL. Table 2 shows the MIC values of both leaf and flower extract. Ethanol Flower extract seem to be more potent than the ethanol leaf extract.

Figure 1. Dose dependent antibacterial activity of Leaf extract of *S.campanulata*

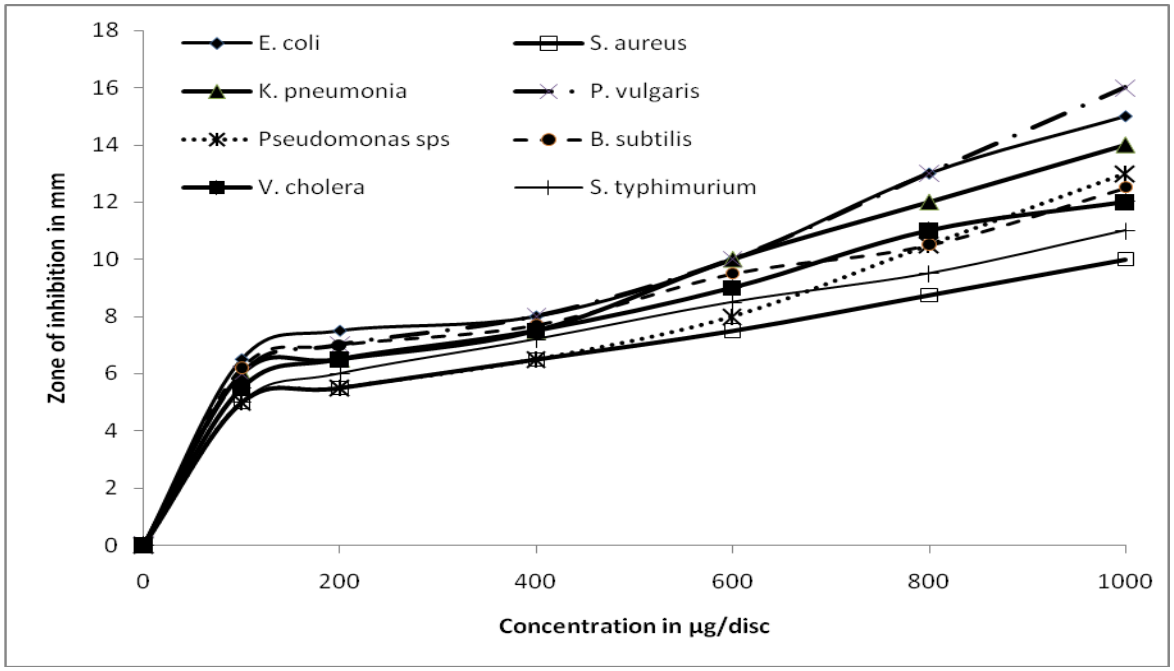

Figure 2. Dose dependent antibacterial activity of flower extract of *S.campanulata*

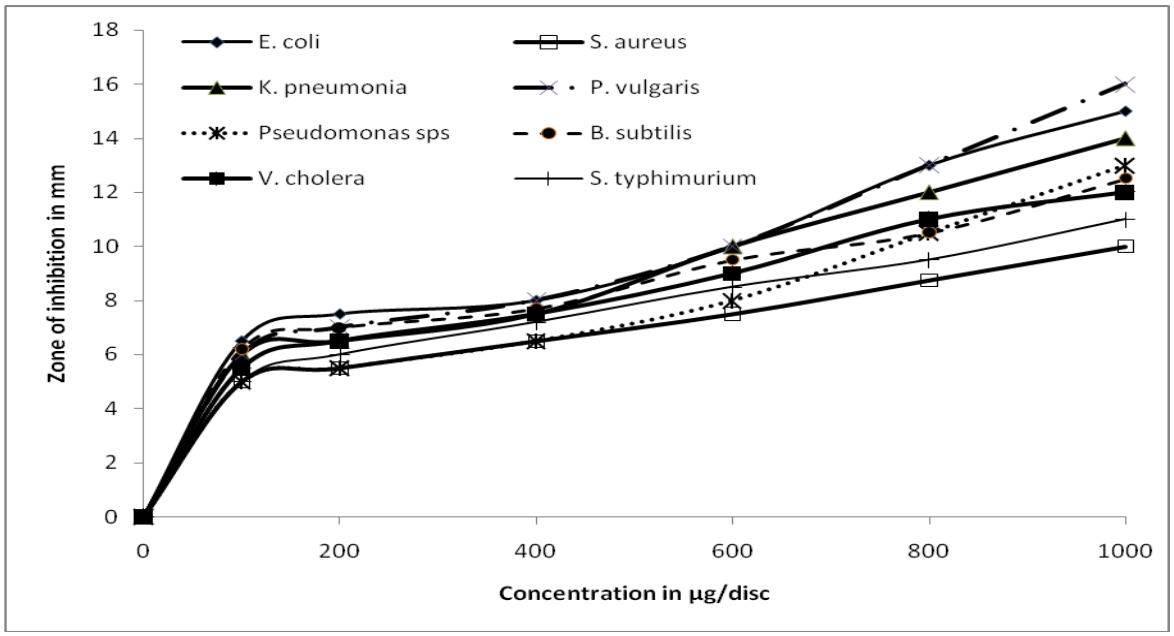

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Table 1. Antibacterial activity of ethanol extracts of *Spathodea campanulata* leaf and flower

Organisms	Zone of inhibition in (mm) at 250 µg/disc		Zone of inhibition in (mm) at 10 µg/disc	
	Spathodea campanulata Ethanolic extract		Standard antibiotic	
	Leaf	Flower	Gentamycin	Streptomycin
Escherichia coli	8.0±0.25	9.6±0.6	26±1	17±0.5
Staphylococcus aureus	6.0±0.5	8.8±0.4	23±0.6	19±1
Klebsiella pneumonia	6.5±0.5	10.5±0.5	21±0.5	17±0.5
Proteus vulgaris	7.2±0.3	10.4±0.4	20±1	14±1
Pseudomonas sps	6.2±0.2	8.6±0.5	24±1	16±0.5
Salmonella typhimurium	6.5±0.4	9.2±0.5	24±1	18±0.5
Bacillus subtilis	7.2±0.2	9.6±0.5	30±0.5	17±2
Vibrio cholera	6.8±0.5	11.2±0.4	23±0.6	18±1

The results are mean ±S.D (n=3).

Table 2. Minimum inhibition concentration of ethanol extracts of *Spathodea campanulata* leaf and flower

Organisms	MIC in µg/ml		MIC in µg/ml	
	Spathodea campanulata Ethenolic extract		Standard antibiotic	
	Leaf	Flower	Gentamycin	Streptomycin
Escherichia coli	221±1.25	168±1.5	20±0.2	13.7±0.5
Staphylococcus aureus	236±1.5	162±1.5	20.8±0.5	16.9±0.3
Klebsiella pneumonia	225±1.5	162±0.5	16.8±0.5	13.7±0.1
Proteus vulgaris	238±0.5	161±1	16±0.2	12.3±0.4
Pseudomonas sps	254±0.5	156±0.5	19.2±0.3	12.6±0.2
Salmonella typhimurium	243±1	173±0.5	19.4±0.3	14.4±0.4
Bacillus subtilis	241±1.5	158±1.5	24.1±0.3	17±0.2
Vibrio cholera	246±1	149±1.5	18.3±0.4	13.8±0.3

The results are mean ±S.D (n=3).

Table 3. Phytochemical screening of *Spathodea campanulata* leaf and flower

Phytochemicals	*Leaf extract*	*Flower extract*
Alkaloids	+ve	+ve
Tannin	+ve	+ve
Saponin	+ve	+ve
Steriod	+ve	+ve
Phlobatannin	-ve	-ve
Terpenoid	+ve	+ve
Flavonoid	+ve	+ve
Phenolics	+ve	+ve
Proteins	-ve	-ve
Glycoside	+ve	+ve

Phytochemical analysis (Table 3) of ethanol extracts showed the presence of alkaloids, saponins, steroids, anthraquinone glycosides, flavonoids, triterpenoids and tannins.

In our study, a wide range of human pathogenic microorganisms were examined, including Gram-positive and Gram-negative bacteria, This may partly indicate that the leaf and flower extracts of *S. campanulata* have broad inhibitory activities to pathogenic microorganisms and are promising to act as potential antibacterial agents from natural plant sources.

Active compounds present in the crude ethanol extracts show the antibacterial activity with the dose dependant manner. If the active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. So this may be happening with the ethanol flower extract where it shows more potency than the leaf extract. Polyphenolic, flavonoids and tannins present in the ethanol extract may be responsible for the antibacterial activity. Tannin is known to show the antibacterial activity by precipitation the microbial proteins. Flavonoids are produced by the plants for the defense against the infection. So, use of the crude ethanol extract of this plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. The findings from this work may add to the overall value of the medicinal potential ethanol extract of leaf and flower extract of *S. campanulata*. Further phytochemical studies are required to determine the purified fractions/bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents.

From the above studies it can be concluded that the ethanol extracts of both Leaf and flower extract of *S. campanulata* exhibit significant antibacterial activity against pathogenic bacteria. The inhibited extracts showed high polyphenols, tannins and flavonoids content. Therefore this *S. campanulata* leaf and flower may be act as another source of natural antibiotic. This study reaffirms the ethanomedicinal property of *S. campanulata.*

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