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THE EFFICACY OF *SPATHODEA CAMPANULATA P. BEAUV* LEAF EXTRACT ON SOME BACTERIAL AND FUNGAL STRAINS BY WELL DIFFUSION METHOD

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ABSTRACT

The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of chloroform, acetone, methanol and aqueous extracts of *Spathodea campanulata.* The antimicrobial activity of different extracts of *S. campanulata* was tested against the Gram-positive and Gram-negative bacterial strains and fungi by agar well diffusion method. The Gram- positive bacteria used in the test were *S.aureus, B.subtilis, B.megaterium, S.mutans* and the Gram-negative bacteria were *E.coli, K.pneumoniae, P.aeruginosa,* fungi like *A.niger, A.flavus, T.viridae and C.albicans.* It was observed that methanol extract exhibited maximum activity against *S. mutans, P.aeruginosa, A. flavus and C.albicans*. Chloroform and acetone extracts were exhibited moderate activity and aqueous extract exhibited less activity against the tested microorganisms. In this study different extracts of leaves of *Spathodea campanulata* exhibited varying degree of inhibition to the growth of tested organisms. The results confirmed the presence of antibacterial and antifungal activity of *Spathodea campanulata* leaf extracts against various human pathogenic microorganisms.

KEYWORDS: ***Spathodea campanulata,*** **phytochemical, antimicrobial, agar well diffusion**

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1. INTRODUCTION

Nowadays antibiotic resistance has become a global concern[1]. The increase in incidence of antibiotic resistance is largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[2,3] . Hence there is a continuous search for effective and safer alternatives. The importance of plant derived material in present day therapy cannot be underestimated. Existing commercial broad spectrum antibiotics have numerous pharmacological drawbacks whereas the drugs of plant origin have no side effects or only marginal. Many herbs are used to treat diseases like cholera, typhoid, diarrhoea, dysentery, urinary tract infections etc., which are caused by the microorganisms. The phytoconstituents like alkaloids, glycosides, saponins, tannins, flavonoids, phenol and phenolic compounds present in the herbs are responsible in curing of the diseases. Rugtoora is an Indian medicinal plant botanically known as *Spathodea campanulata* belongs to the family *Bignoniaceae* (Figure:1). *Spathodea* is a monotypic genus in the flowering plant commonly known as the Fountain Tree, African Tulip tree, Pichkari or Nandi Flame[4]. It is native to tropical dry forests of Africa. This is an ornamental tree with very showy reddish-orange or crimson flowers. Apart from having decorative and attractive features, it has some folkloric medicinal uses. The dried, pulverized bark or fresh inner bark of the plant is used as a dressing for ulcers and other skin problems. The Indian people use a decoction of the leaves and bark as a soothing lotion. The decoction of bark is usually given for treating the diseases like dysentery, gastrointestinal and kidney troubles. The infusion of leaves is used in the treatment of urethral inflammation and as well as antidote for animal poison. The flowers are employed as diuretic and anti-inflammatory. The leaf and flower ethanol extracts of *Spathodea campanulata* were investigated for antimicrobial activity and it was reported that the flower extract was more potent than leaf extract[5]. The ethanol extract of leaf possess anticonvulsant activity[6]. The ethanol leaf extract of *S.campanulata* was investigated for analgesic and anti-inflammatory potentials[7]. The phenolic derivatives present in *S.campanulata* roots produced fungitoxic properties[8]. The present study was carried out to test the antimicrobial efficacy of the leaves extracts of *Spathodea campanulata* against human pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

1.1 Plant Material

The fresh leaves of *S. campanulata* were collected in and around Guntur, Andhra Pradesh, India. The plant was identified and authenticated by Dr. M. Raghu Ram, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. The healthy leaves were shade dried and powdered using electric blender to get a coarse powder.

1.2 Extraction

The powdered leaf material was extracted with selected solvents chloroform, acetone and methanol by cold maceration process[9]. The extracts were prepared by imbibing 30g of powder with 200mL of each solvent in separate containers and kept in a shaker for 24 h. The aqueous extract was prepared by boiling 30g of powder with 200mL at mild temperatures. The extracts were collected by filtration through 5 layers of muslin cloth. The extraction process was repeated twice. The collected filtrates were pooled, concentrated and dried at mild temperature. The prepared extracts were preserved in dessicator for further study.

1.3 Phytochemical Screening

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 been isolated from the stem bark [10-14]. The

flowers of *S. campanulata* possess anthocyanins[15]. The preliminary phytochemical screening for the extracts was carried out by standard protocol[16,17].

1.4 Antimicrobial activity

The present study was designed to determine the susceptibility pattern of various compounds of *S.campanulata* utilizing a broad spectrum of pathogenic bacteria like *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Streptococcus mutans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and fungi like *Aspergillus niger, Aspergillus flavus,Trichoderma viridae and Candida albicans*. All the stock cultures were obtained from Department of Microbiology, Acharya Nagarjuna University, Guntur. The stock cultures were preserved in refrigerator for further study.

1.5 Preparation of sample solution

The stock solution of chloroform, acetone, methanol and aqueous extracts were prepared at the concentration of 20mg/ml using dimethyl sulphoxide (DMSO). Stock solutions were diluted with DMSO to get concentrations of 600 µg and 800 µg per well. The standard antibiotics (Ciprofloxacin & Griseofulvin) solutions were prepared at the concentration of 1mg/ml using DMSO. The standard antibiotics at concentration of 10 µg were used as reference standard. The solvent DMSO was used as control.

1.6 Culture media and inoculums preparation

Nutrient agar and broth (Himedia, India) were used as the media for the culturing of bacterial strains. Potato dextrose agar and broth (Himedia, India) were used as the media for the culturing of fungal strains. The antimicrobial activity was evaluated by agar well diffusion method[18,19]. The organisms grown in broths were used as inoculums. 0.1 ml of diluted inoculum (10^5 CFU/ml) of selected test organisms were spread on nutrient agar and potato dextrose agar plates. The plates were allowed to dry and wells of 6 mm diameter were punched equidistantly into the agar medium. The activity was determined in dose dependent manner.

1.7 Testing for Antibacterial Activity

The extracts prepared were screened for their antibacterial activity in comparison with standard antibiotic ciprofloxacin by well diffusion method. The antibacterial activity of the crude extracts was performed by the cup-plate agar diffusion method . The wells were filled with 600 µg and 800 µg concentrations of chloroform, acetone, methanol and aqueous plant extracts respectively. The antibacterial activity was compared with ciprofloxacin at a concentration of 10 µg.

1.8 Testing for Antifungal Activity

The extracts prepared were screened for their antifungal activity in comparison with standard antifungal drug griseofulvin by well diffusion method. Lawn culture was prepared using the test organisms grown in potato dextrose broth. The cup-plate agar diffusion method was employed to assess the antifungal activity of the prepared crude extracts. The prepared wells were filled with 600 µg and 800 µg concentrations of chloroform, acetone, methanol and aqueous plant extracts respectively. The antifungal activity was compared with griseofulvin at a concentration of 10 µg.

2. RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Spathodea campanulata* leaves showed the presence of alkaloids, steroids, cardiac glycosides, tannins and phenolic compounds. The results were reported in table-1. Biological screening of plant extracts are most frequently carried out as determination of antimicrobial activity. These evaluations are done by means of standard *in vitro* assays (agar well diffusion or Tube dilution) utilizing a broad spectrum of pathogenic and non-pathogenic bacteria. The results of antibacterial activity were reported in table-2, which clearly show that all the extracts have shown good antibacterial activity against the tested Gram-positive and Gram-negative

bacteria. Chloroform extract was more effective against *S.mutans.* Acetone extract was more effective against *S.mutans, P.aeruginosa* and *K.pneumoniae*. Methanol extract was more effective against *S.mutans* and *P.aeruginosa*. Aqueous extract was more effective against *S.mutans.* The results of antifungal activity were reported in table-2, which clearly show that all the extracts have shown good antifungal activity against all the tested fungal species. Chloroform and extracts were more effective against *T.viridae.* Methanol extract was more effective against *A.flavus and C.albicans.* Aqueous extract was more effective against *A.niger*. The maximum antimicrobial activity was exhibited by methanol extract against *S.mutans, P.aeruginosa* and *A.flavus.* Chloroform and acetone extracts were exhibited moderate activity and aqueous extract exhibited less activity against the tested microorganisms.

Table-1

Preliminary Phytochemical Screening of leaves of Spathodea campanulata

Tests	Chloroform	Acetone	Methanol	Aqueous
Carbohydrates	+	–	–	–
Gums	–	–	–	–
Mucilage	+	+	+	+
Proteins	–	–	–	–
Aminoacids	–	–	–	–
Fats & Oils	+	–	–	–
Steroids	–	–	–	+
Cardiac glycosides	–	–	–	+
Flavonoids	–	–	–	–
Alkaloids	+	+	+	–
Tannins & phenolic compounds	–	+	+	+
Vitamins	–	–	–	–

'+' indicates positive, '–' indicates negative

Table-2

Antimicrobial Activity of leaves of Spathodea campanulata

Name of the organism	Zone of inhibition in mm										
	Chloroform		Acetone		Methanol		Aqueous		DMSO	CP	GF
	600 µg	800 µg	600 µg	800 µg	600 µg	800 µg	600 µg	800 µg	10 µl	10 µg	10 µg
S. aureus	8	10	9	12	9	12	8	9	Nil	33	ND
B. subtilis	8	10	10	12	11	13	8	9	Nil	34	ND
B.megaterium	8	10	9	11	10	12	8	8	Nil	33	ND
E. faecalis	8	10	9	11	9	11	8	10	Nil	32	ND
S. mutans	9	12	10	13	11	14	9	12	Nil	31	ND
E. coli	8	10	9	12	10	13	8	10	Nil	32	ND
P. aeruginosa	9	11	11	13	11	14	8	10	Nil	31	ND
K.pneumoniae	8	10	9	13	9	13	8	11	Nil	32	ND
A. niger	8	10	8	10	8	10	8	9	Nil	ND	26
A. flavus	8	10	10	11	10	12	Nil	8	Nil	ND	28
T. viridae	9	11	10	12	9	11	Nil	8	Nil	ND	29
C.albicans	10	11	11	11	11	12	8	9	Nil	ND	29

CP – Ciprofloxacin, GF – Griseofulvin

Figure 1
Spathodea campanulata

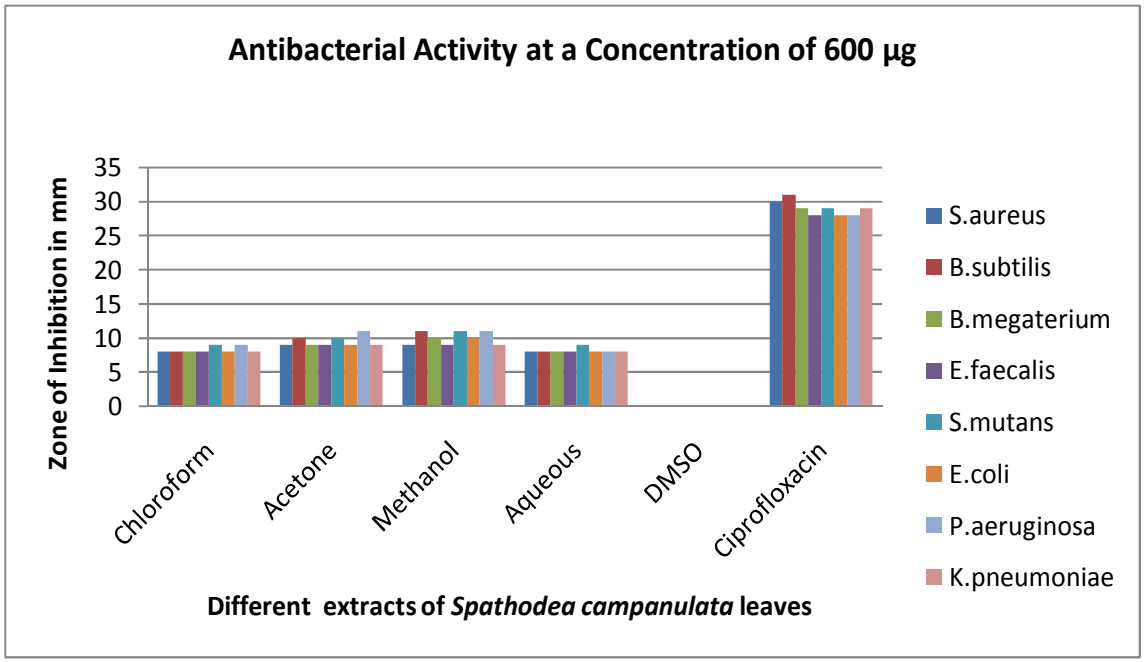

Figure 2

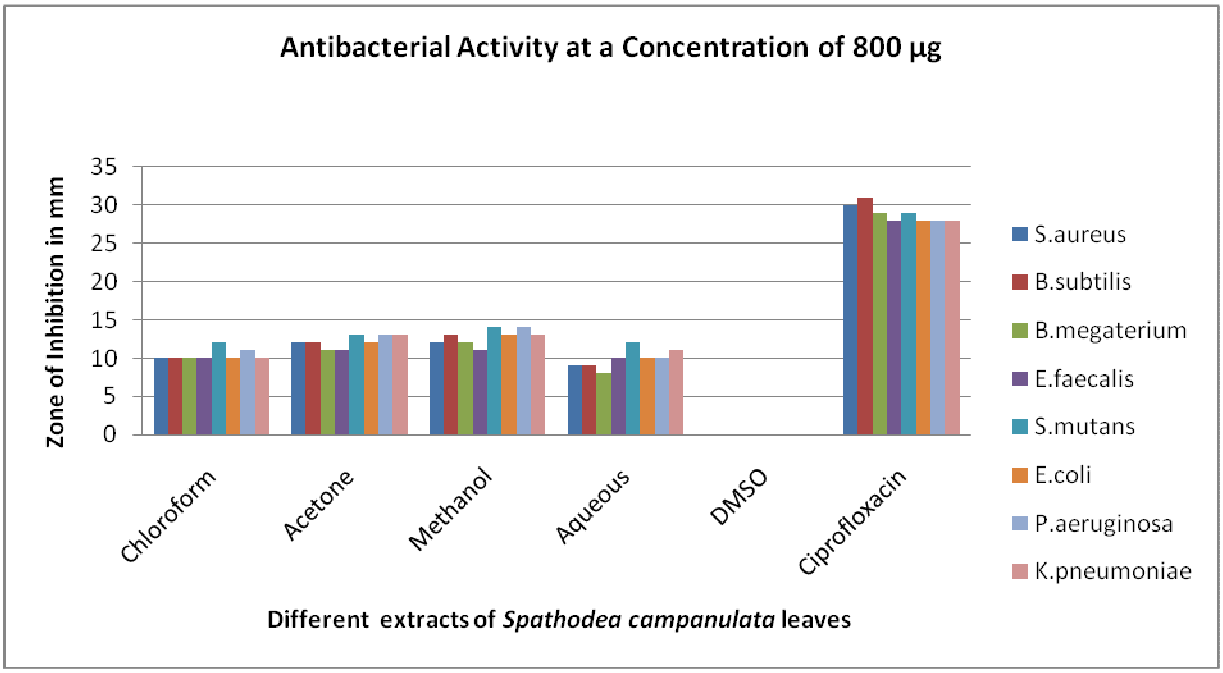

Figure 3

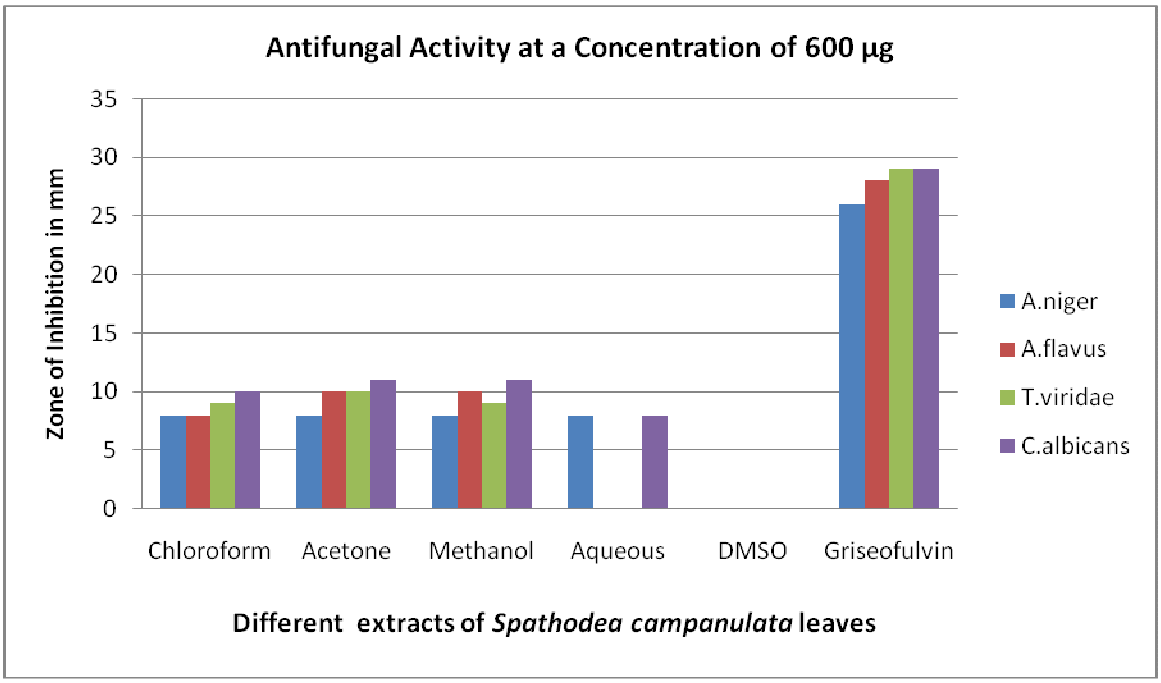

Figure 4

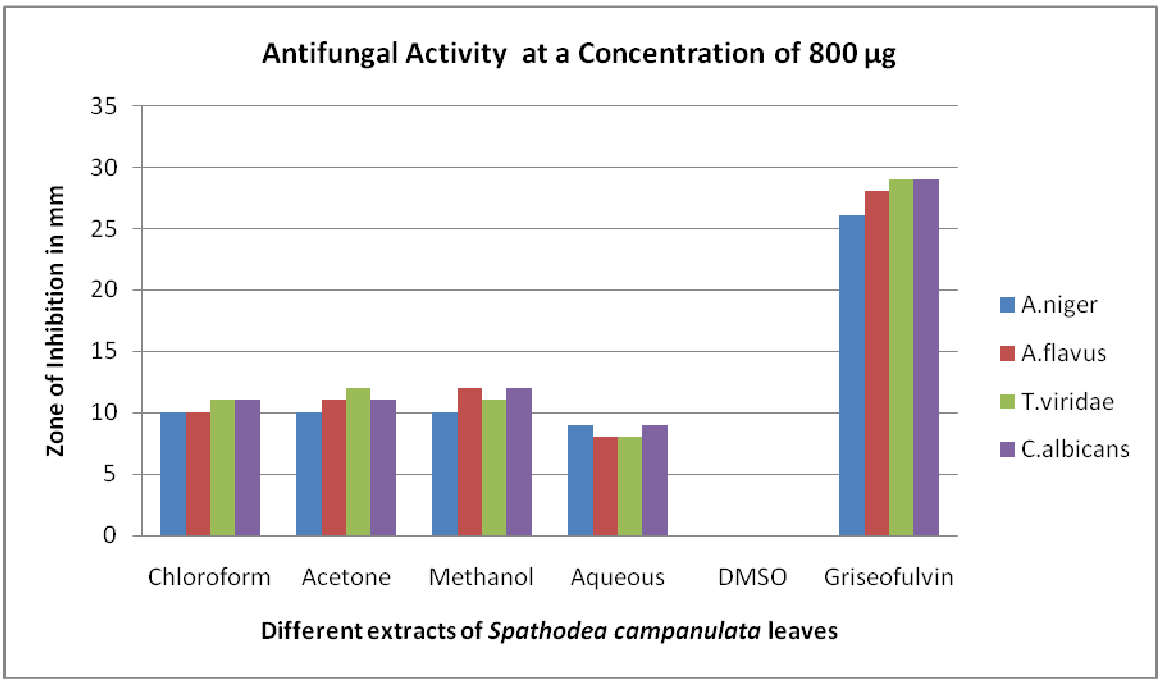

Figure 5

3. CONCLUSION

The results reported in the present work shows evidence that the leaf extracts of *Spathodea campanulata* possess antimicrobial activity. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections. Isolation, Identification and purification of the phytoconstituents and determination of their respective antimicrobial potencies are helpful in formulating novel chemotherapeutic agents and it should be the future direction for investigation.

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