

***In vitro* Phytochemical Screening and Antibacterial Activity of Organic Leaf Extracts of *Spathodea campanulata* P. Beauv against Hospital Isolated Bacterial Strains**

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Issued 17 November 2008

ABSTRACT

Spathodea campanulata P. Beauv is extensively used in Indian traditional and folklore medicines to cure various human ailments. The preliminary phytochemical screening of the leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids and terpenoids. *In vitro* antibacterial studies of the leaf extracts were carried out on eight medically important bacterial strains using agar disc diffusion method. The antibacterial assay using petroleum ether leaf extracts showed good inhibitory activity against *Klebsiella pneumoniae* and compared with standard antibiotic Streptomycin.

Key words: *Spathodea campanulata*, Phytochemical Screening, Leaf extracts and Antibacterial activity.

INTRODUCTION

Infectious diseases are the leading cause of death worldwide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.*, 2003). Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Scazzocchio *et al.*, 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-Seedi *et al.*, 2002; Rojas *et al.*, 2003; Duraipandiyani *et al.*, 2006; Parekh and Chanda, 2007a). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. The results of investigation performed in the

late 19 and 20 century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man (Asirvatham Doss and Rangasamy Dhanabalan., 2008).

Spathodea campanulata is a species belonging to the Bignoniaceae family, native from equatorial Africa. The Siddha/Tamil name of this species is Patadi and in folk it is popularly called as Ruugatuuraa. It is very commonly found and planted in the coffee estates of Munnar, South Tamilnadu and denoted by the name Malaria Maram(tree). In English the species is called as Syringe tree, Fountain tree, African Tulip tree, Flame-of-the-forest or Nandi Flame. It is a medium-size tree (15-25 m high), characterized by red garish flowers. It is often employed in gardening in tropical and subtropical areas including South America (Joly, 1985). Flowers and stem bark extracts have shown molluscicidal activity and also employed in diuretic, anti-inflammatory treatments. The leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches and diarrhea (Jardim *et al.*, 2003; Mendes *et al.*, 1986). Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts (Niyonzima *et al.*, 1999; Makinde *et al.*, 1988). Preliminary *in vitro* antimalarial activity of *Spathodea campanulata* evaluated (Rangasamy Dhanabalan *et al.*, 2008).

Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, leaves, flowers and fruits (Ngouela *et al.*, 1990; Ngouela *et al.*, 1988; Amusan *et al.*, 1995; Amusan *et al.*, 1996). The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids (Ngouela *et al.*, 1991; Subramanian *et al.*, 1973; El-Hela, 2001a; El-Hela, 2001b). Banerjee and DE (2001) showed the presence of anthocyanins in flowers of *S. campanulata*. A qualitative fungitoxic activity of *S. campanulata* roots against *Cladosporium herbarum* CCT 0279 has been evaluated and reported (Pianaro *et al.*, 2007). *In vitro* antibacterial activity of leaf extracts of this plant against standard strains was evaluated (Parek.J and Chanda.S, 2007). Thus, the present work is aimed to evaluate the antibacterial activity of *S. campanulata* leaf extracts against the clinically isolated medically important species.

MATERIAL AND METHODS

Plant materials

Fresh plant leaf samples were collected from the Munnar Kundala Tea Estates, Kerala, India during March 2008. The taxonomic identity of the plant was confirmed by Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India and the voucher specimen of the plants was preserved in Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamilnadu, India. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder and stored in airtight bottles.

Preparation of plant extract

About 100g of the powder was extracted with different organic solvents viz, Benzene, Chloroform, Methanol, Petroleum ether and Water and allowed to stand overnight. The extract was filtered through Whatman no.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure.

Bacterial Strains

The antibacterial activities of the extracts were tested using hospital isolated Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus citreus*, *Bacillus subtilis* and Gram negative bacteria: *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia sp.*, and *Proteus vulgaris*. All the strains were procured from Department of Microbiology, RVS Hospital, Coimbatore, Tamilnadu.

Phytochemical Screening Test

The phytochemical screening of the plant extract was carried out by following methods used by Amarasingham *et al.*, (1964), Das and Bhattacharjee, (1970), Gibbs, (1974), Trease and Evans (1978), Santaram and Harborne (1984) to detect the presence or absence of certain bioactive compounds:

Anti bacterial assay

Three different concentrations of the leaf extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of Bauer *et al.*, 1966. The strains obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37⁰C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), dispensed on petri dishes and lawn cultures were prepared using sterile cotton swabs with the test organisms from the seeded broth. Sterile discs of six millimeter width impregnated with 20 µl of test extract in different concentrations were placed on the upper layer of the lawn cultures. Streptomycin (10µg/disc) was used as standard. The plates were incubated overnight at 37⁰C. Antibacterial activity of the plant extract was assayed by measuring the inhibition zone formed around the discs. The experiment was repeated triplets and the mean values were calculated.

RESULTS

The preliminary phytochemical analysis of the leaf extract revealed the presence of Alkaloids, Flavonoids, Steroids, Saponins, Terpenoids and Tannins (Table 1). The results obtained from the disc diffusion assay showed an increasing inhibitory effect on bacterial growth with increasing concentration of the extract. The extracts showed inhibitory activity on almost all bacterial strains tested. Among all the tested organisms, the gram negative bacterial strain, *K.pneumoniae* was found to be more susceptible to the plant extract with an inhibition zone ranging from 11 mm and the gram positive strains were least susceptible with the inhibition zone ranging from 10 mm. The antibacterial activity in terms of zone of inhibition was presented in Table 2. The observed activity may be due to the presence of potent phytochemical constituents in the leaf extracts.

DISCUSSION AND CONCLUSION

Plants have been a veritable source of drugs. However, man tends to ignore the importance of herbal medicine. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade (Patrick Ekong Ebong, *et al.*, 2008). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Veeramuthu Duraipandiyan *et al.*, 2006). According to World Health Report of

Infectious diseases 2000; overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management.

Antibacterial property of Ethanol and Methanol leaf extracts of *Spathodea campanulata* was already proved against standard strains of *Klebsiella pneumonia* (Parekh and Chanda.S, 2007). The same result was proved with petroleum ether and methanol leaf extracts of *Spathodea campanulata* in our study. The antibacterial activity of petroleum ether leaf extracts showed better result and the strains used in the study were purely isolated from hospital environment. Further studies are essential for the isolation of the therapeutic antimicrobials and carry out pharmacological evaluation of *Spathodea campanulata*.

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Table 1. The phytochemical profile of the leaf extracts of *Spathodea campanulatum*.

S.No	Secondary Metabolites	P.ether	Benzene	Chloroform	Methanol	Water
1	Alkaloids	+	-	-	-	-
2	Flavanoids	-	-	+	+	-
3	Cardiac glycosides	-	-	-	-	-
4	Steroids	-	-	+	+	-
5	Saponins	-	-	-	+	+
6	Terpenoids	-	-	-	+	-
7	Tannins	-	-	-	+	-

Table 2. Antimicrobial activity of *Spathodea campanulatum* Leaf extracts.

S.No	Microorganism	Concentration of extracts/Zone of Inhibition (mm)				
		Petroleum ether	Benzene	Chloroform	Methanol	Water

		(mg/ml)			(mg/ml)			(mg/ml)			(mg/ml)			(mg/ml)		
		10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
1	<i>S. aureus</i>	10	9	7	9	7	-	9	7	-	9	8	-	9	7	-
2	<i>S. Citrus</i>	9	8	-	7	-	7	-	-	-	-	-	-	-	-	-
3	<i>B.subtilis</i>	10	7	-	9	8	-	-	-	-	-	-	-	-	-	-
4	<i>Serratia</i>	8	9	8	9	8	9	9	7	-	10	7	-	9	7	-
5	<i>P.vulgaris</i>	10	9	8	8	7	-	10	9	7	9	8	-	10	7	-
6	<i>K. pneumoniae</i>	11	9	8	8	7	-	-	-	-	4	3	1	-	-	-
7	<i>S. Typhi</i>	9	8	-	8	7	-	10	9	-	10	9	7	10	9	7
8	<i>P.aeruginosa</i>	9	7	-	8	-	-	9	8	7	9	8	7	9	8	7

mm- Zone of inhibition in millimeter, mg-Concentration of the leaf extracts in organic solvents.