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Preliminary Phytochemical Screening and Antibacterial Studies of Leaf Extract of *Solanum trilobatum* Linn.

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Abstract

Solanum trilobatum Linn. 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 Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycoside, Terpenoids and Lipids. In vitro antibacterial studies on the leaf extracts were carried out on eight medically important bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococccus* pyrogens, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Proteus vulgaris* which were procured from Department of Microbiology, RVS Hospital, Sulur,Coimbatore, Tamilnadu., using agar disc diffusion method. The bacterial strains were exposed to the following four different concentrations of extracts: 25mg/ml, 10mg/ml and 5mg/ml solvent. The results of our antibacterial assay revealed that the extract showed good inhibitory activity against all the tested pathogens compared with standard antibiotics like streptomycin. The inhibitory activity was found to be dose dependent.

Introduction

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti, A et al., 2008). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube N. S.., 2008).

Solanum trilobatum (Solanaceae – herbs) is an important medicinal plant. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates, protein, fat, crude fibre, and minerals (Jawahar *et al*., 2004).This herbal plant is used as medicine for asthma, vomiting of blood, reducing blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy. It is also antibacterial, antifungal antimitotic, antioxidant and antitumouours (Subramanian and Madhavan, 1983, Shahjahan *et al.*, 2005, Shahjahan *et al*., 2004, Purushothaman *et al*., 1969 and 1972).

MATERIALS AND METHODS

Collection of plant materials

Mature leaves were collected from the healthy plant at Nilgiris, Tamilnadu, India. The collected plant materials were botanically authenticated by Botanical Survey of India (Southern Circle),Coimbatore, Tamilnadu.

Preparation of plant extract

The leaves were washed in tap water, shade dried for 10 days and made into a fine powder of 40 mesh size using the laboratory mill. Following that, 100g of the powder was extracted with different organic solvents viz, Chloroform, methanol, petroleum ether and water and it was stand for over night. 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.

Test organisms

The extract was tested on the following three Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococccus* pyrogens . Five Gram negative bacteria were also tested, including *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Proteus vulgaris* . All the strains were procured from Department of Microbiology, RVS Hospital, Coimbatore, Tamilnadu.

Phytochemical screening

Phytochemcial analysis of the extract was conducted by Trease and Evans.,1989 : Harborne (1998). By this analysis, the presence of several phytochemicals like Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycoside, Terpenoids and Lipids were tested.

Anti bacterial assay

The 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 of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37^0 C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0 C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) was used as standards.

RESULTS

The preliminary phytochemical analysis of the leaf extract revealed the presence of Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycoside, Terpenoids, Lipids as presented in Table 1. The results obtained from the disc diffusion assay showed that there has been an increasing effect on bacterial

growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the Gram negative bacterial strain, *Bacillus subtilis*, was found to be more susceptible to the plant extract by showing inhibition zone ranging from 18 mm and the gram negative strain *Staphylococcus epidermis* was least susceptible with the inhibition zone ranging from 20 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 phytoconstituents in the leaf extracts.

DISCUSSION AND CONCLUSION

Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, *Ayurveda*. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (R. Nair, et al., 2005). 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. *Solanum trilobatum* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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Table 1. The phytochemical profile of the leaf extract.

Phytochemcials	Presence/Absence
Sugar	+
Protein	+
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Cardiac Glycoside	-
Terpenoids	+
Lipids	-

Table 2. Antibacterial activity of leaf extracts of *Solanum trilobatum*.

Microorganisms	Zone of inhibition including disc diameter (mm)								
	Chloroform				Methanol				Str
	a	b	c	MIC	a	b	c	MIC	
Bacillus subtilis	18	13	9	1	12	10	9	2	22
S.aureus	10	8	-	2	15	13	8	1	16
S.pyogenes	21	17	11	0.125	16	12	9	1	20
E.coli	14	9	-	2	10	9	-	2	21
K.pneumoniae	10	8	-	2	12	10	-	2	15
P.aeruginosa	16	10	9	1	18	14	10	1	17
P.vulgaris	12	10	9	2	14	11	-	1	14
S.typhi	14	11	9	1	20	16	10	0.5	20

Microorganisms	Zone of inhibition including disc diameter (mm)								
	Petroleum ether				Water				Str
	a	b	c	MIC	a	b	c	MIC	
Bacillus subtilis	18	16	10	1	11	10	-	4	22
S.aureus	15	10	9	2	18	13	10	2	16
S.pyogenes	18	13	10	1	15	13	10	1	20

E.coli	10	9	-	2	12	10	-	2	21
K.pneumoniae	12	9	-	2	14	13	10	2	15
P.aeruginosa	17	15	11	1	1	16	9	1	17
P.vulgaris	14	10	-	2	14	11	12	2	14
S.typhi	16	12	10	1	19	14	9	1	20

a, b, c indicates 25, 10, and 5 mg/ml concentrations, respectively. MIC denotes Minimum Inhibitory Concentration. Str- indicates Streptomycin.

All the values are mean ± standard deviations of three determinations.