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STUDIES ON ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF SOLANUM TRILOBATUM AGAINST SOME HUMAN PATHOGENS

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Abstract

The *in vitro* antibacterial activity, minimal inhibitory concentration (MIC) and phytochemical screening of principle bioactive compounds in *Solanum trilobatum* (leaf, fruit and root) extracts were evaluated for their antibacterial activity against some human pathogens *viz*., *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. MIC studies were carried out with *Staphylococcus aureus* and *Klebsiella pneumoniae* on the basis of potentially inhibitory effect against plant extracts by disk diffusion method with chloramphenicol and solvents (aqueous, methanol and chloroform) served as positive and negative controls, respectively. All solvent extracts inhibited the growth of tested microorganisms. Among the solvent extract tested, methanol extract showed maximum antibacterial activity against all the organisms when compared to aqueous and chloroform extract. *Solanum trilobatum* leaf extract found to possess maximum antibacterial activity than fruit and root extracts. *Staphylococcus aureus* were found to be more susceptible to the tested plant extracts than *Klebsiella pneumoniae* and inhibited at relatively lower concentration of about 20 mg mL^{-1}. Phytochemical analysis revealed the presence of components such as alkaloids, saponin, flavonoids, anthroquinone and trepenoid indicates *S. trilobatum* is one of the potential medicinal plant for therapeutic use. The results of the present study clearly envisaged the antibacterial activity of *Solanum trilobatum* and phytochemicals present in the plant extracts may have acted alone or in combination were effective against tested pathogenic microorganisms.

Key Words: *Solanum trilobatum*, antibacterial activity, disk diffusion method, minimal inhibitory concentration (MIC), phytochemical analysis, zone of inhibition.

INTRODUCTION

Phytopharmaceuticals are an inexhaustible reservoir of chemotherapeutics to treat many ailments such as cold, fever, diarrhoea, psychic problems, birth control and dental hygiene throughout the world (Mitscher *et al*., 1987). It is estimated that there are 2,50,000 to 5,00,000 species of plant species are believed to exist on earth. India, owing to its vast green forests, rivers and hills, with its richness of biodiversity can be considered as the paradise of medicinal plants. Medicinal plants are very important source of life saving drugs for the ever increasing world population. The developing countries greatly depend on plants, where a major role in health care is played by traditional medicine (Zakaria, 1991). The use of synthetic drugs leads to undesirable hazards and side effects. India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.

In the modern world, multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on body (Chowdhury, 2002;

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Wang, 2002; Lewis and Ausubel, 2006).

The genus *Solanum* was established by Carl Linnaeus in 1753. *Solanum trilobatum* L. (Solanaceae) is a rare, a thorny creeper with bluish violet flower, perennial, medicinal herb found in some of the warmer parts of the tropical and subtropical regions. Popularly called 'thuduvalai' by the local tribes, villagers and herbalists, this ethnobotanical herb is known to have unique medicinal properties (Mohan and Devi, 1998). The Siddha system of medicine uses a ghee prepared from this plant for treatment of tuberculosis.

Solanum trilobatum (Solanaceae herbs) possess some amount of calcium, iron, phosphorus, carbohydrates, fat, crude fiber and minerals in the leaves (Doss *et al*., 2009). Preparations made from the leaves and stems of the plant are used in herbal medicine for asthma, chronic febrile affections, difficult parturition, arrest blood vomiting, to reduce blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy. It also found to possess antibacterial, antifungal, antioxidant and anti-tumour properties (Jawahar *et al*., 2004; Sugnanam *et al*., 2015). The active principle (Sobatum) obtained from the petroleum ether extract of the plant was proved to be an anticancer agent by *in vitro* and *in vivo* experiments. The present study was carried out with the aim to evaluate the antibacterial activity, minimal inhibitory concentration (MIC) and phytochemical analysis of medicinal herb, *Solanum trilobatum*.

MATERIALS AND METHODS

Collection of plant

Healthy leaves, fruit and root of *Solanum trilobatum* L. were collected from Sadupperi village of Thiruvannamalai district, Tamil Nadu, India.

Preparation of plant extracts

The plant materials like leaf, fruit and root were washed thoroughly with tap water and then with sterile distilled water for the removal of dust and sand particles. The leaf, fruit and root were shade dried and powdered by hand crushing. The powdered samples were then mixed in 1:5 ratios with solvents *viz*., aqueous, methanol and chloroform, separately. The contents were incubated as such in room temperature for 48 h with constant agitation at regular intervals. After 48 h, the contents were filtered through Whatmann No. 1 filter paper followed by Wong *et al*., (1994) with slight modification. The filtrates were vacuum dried using rotary evaporator and concentrates were stored at 4°C. The residues were redissolved with the appropriate solvents from which they were prepared and used for the antibacterial assay.

Test organisms

Staphylococcus aureus, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, Rajah Muttiah Medical College and Hospital (RMMCH), Annamalai University, Chidambaram were used for the present study. The microorganisms were maintained on nutrient agar slants at 4°C.

In vitro antibacterial assay

The disk diffusion method was employed for determining antibacterial activity of *Solanum trilobatum* L. leaf, fruit and root extracts as described by Bauer *et al*. (1966). The 4 to 6 h old cultures were used for the bioassay. Each 4 to 6 h old bacterial cultures were suspended in 2 mL of sterile normal saline and the turbidity of this suspension was adjusted to a 0.5 Mc Farland standard to yield 2.4×10^8 CFU mL^{-1} using turbidometer. A sterile cotton swab were dipped in the cultures and swabbed over the surface of Mueller Hinton agar. The cultures were allowed to dry for 5 min and the sterile filter paper discs (5 mm) with 50 µL of known concentration of plant extracts were impregnated over the surface of Mueller Hinton agar medium using sterile forceps. Chloramphenicol and solvent discs (aqueous, methanol and chloroform) were used as positive and negative control, respectively (Parekh and Chanda, 2007). These plates were then incubated at 37°C for 24 h and the diameter of the zone of inhibition was measured and recorded in millimeter.

Determination of minimal inhibitory concentration (MIC)

MIC test was carried out by agar disk diffusion method. On the basis of results obtained by disk diffusion method, two bacterial isolates *viz*., *Staphylococcus aureus* and *Klebsiella pneumoniae* were selected for MIC studies because of their potential inhibitory effect against the plant extracts when compared to other bacterial isolates. The MIC of plant extracts of *Solanum trilobatum* L. leaf, fruit and root was determined as described by Greenwood (1989). The different concentrations of each extract were prepared such as 20 mg mL^{-1}, 30 mg mL^{-1}, 40 mg mL^{-1}, 50 mg mL^{-1} and 60 mg mL^{-1} were added to the filter paper discs. After drying, apply the discs

using sterile forceps by impregnating discs with centres at least 24 mm apart. The Chloramphenicol and solvent discs (aqueous, methanol and chloroform) were used as positive and negative control, respectively. Then, the plates were sealed with parafilm and incubated at 37 °C for 24 h at inverted position. After incubation, the minimum inhibitory concentration of extracts can be determined by the zone of inhibition formed with lowest concentration.

Phytochemical analysis of Solanum trilobatum

Standard procedures were followed to identify the chemical constituents in aqueous, methanol and chloroform extracts of *Solanum trilobatum* leaf, fruit and root. Qualitative tests for alkaloids, carbohydrates, glycosides, flavonoids, treponoids, tannins, saponins, protein and anthroquinone were performed according to the procedure as described by Harborne (1973) and Sofowara (1993).

Statistical analysis

Samples were compared using one way analysis of variance (ANOVA) using SPSS ver. 12.0.1 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

The *in vitro* antibacterial activity of aqueous, methanol and chloroform extracts of *Solanum trilobatum* leaf, fruit and root was assayed by disc diffusion method (Table 1). The results obtained from the disc diffusion assay showed that the plant extracts possess antibacterial activity against the tested organism. It was found that inhibitory activity of three solvent extracts varies with the tested pathogenic microorganisms. Among the solvent extract tested, methanol extract showed maximum antibacterial activity against all the organisms when compared to aqueous and chloroform extracts. The aqueous extracts were found to possess least antibacterial activity among the solvent extracts. *Staphylococcus aureus* were found to be more susceptible when compared to other isolates *viz*., *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The leaf extracts were found to possess maximum antibacterial activity followed by root and fruit extracts.

Minimal inhibitory concentration of *Solanum trilobatum* L. leaf, fruit and root extracts were tested against two pathogenic microorganisms *viz*., *Staphylococcus aureus* and *Klebsiella pneumoniae* were more susceptible to the solvent extracts when compared to other pathogenic microorganisms and results are shown in Table 2. *Staphylococcus aureus* were found to be more susceptible to the plant extracts *viz*., *Solanum trilobatum* leaf, fruit and root extracts, since their growth was inhibited at relatively lower concentration (20 mg mL^{-1}) than *Klebsiella pneumoniae*. The plant extract, *S. trilobatum* leaf extract inhibited the tested pathogenic microorganisms *viz*., *Staphylococcus aureus* and *Klebsiella pneumoniae* with lowest concentration to a maximum level than other extracts. The *S. trilobatum* fruit extract were found to possess least MIC values and root extract were found to be intermediary in their action. The methanol extracts inhibited pathogenic microorganisms with lower concentration than aqueous and chloroform extracts.

The results of qualitative phytochemical analysis of *S. trilobatum* leaf, fruit and root extracts revealed the presence of alkaloids, saponin, flavonoids, anthroquinone and trepenoid, etc (Table 3). In methanolic extracts of *S. trilobatum* leaf, fruit and root, alkaloids, flavonoids, trepenoid, saponin and

Table 1 Antibacterial activity of *Solanum trilobatum*

Test organism	Zone of inhibition in mm									Chloramphenicol (Positive control)
	Leaf			Fruit			Root			
	A	M	C	A	M	C	A	M	C	
Staphylococcus aureus	12±0.75	19±0.33	8±0.14	10±0.88	17±0.29	8±0.63	10±0.65	11±0.42	9±0.10	26±0.43
Bacillus subtilis	8±0.47	13±0.00	6±0.71	-	12±0.21	7±0.38	-	-	-	24±0.66
Klebsiella pneumoniae	11±0.35	17±0.91	7±0.22	9±0.00	15±0.80	-	-	9±0.73	-	20±0.72
Proteus vulgaris	8±0.00	11±0.87	-	-	10±0.73	-	-	-	-	21±0.94
Pseudomonas aeruginosa	9±0.08	10±0.29	-	-	9±0.52	-	-	-	-	16±0.22

A – Aqueous; M – Methanol extract; C – Chloroform extract; '-' – Absent

Table 2 Minimal Inhibitory Concentration (MIC) of various extracts of *Solanum trilobatum*

Organism	Name of the solvent	Concentration in mg mL^{-1}											
		Leaf				Fruit				Root			
		20	30	40	50	20	30	40	50	20	30	40	50
Staphylococcus aureus	Aqueous	+	+	+	-	+	+	+	-	+	+	+	+
	Methanol	-	-	-	-	+	-	-	-	+	+	-	-
	Chloroform	+	-	-	-	+	-	-	-	+	+	-	-
Klebsiella pneumoniae	Aqueous	+	+	+	-	+	+	+	-	+	+	+	+
	Methanol	+	-	-	-	+	-	-	-	+	+	-	-
	Chloroform	+	-	-	-	+	+	-	-	+	+	+	-

'+' – Growth appeared; '-' – No growth appeared

anthroquinone were found to present than other solvent extracts. The phytochemicals were majorly extracted in methanol, when compared to aqueous and chloroform. When comparing among plant parts, leaf were found to contain various number of phytochemicals than fruit and root.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The increased frequency of resistance to commonly used antibiotics led to search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases and plants belonging to the *Solanum* genus have been reported to have remarkable pharmacological activity (Mahadev *et al*., 2014). In the present study, our results clearly envisaged the antibacterial activity of *S. trilobatum* (leaf, fruit and root). The antibacterial activity of methanol extracts was more pronounced than the aqueous and chloroform extract against the tested

Table 3 Phytochemical analysis of *Solanum trilobatum*

Secondary metabolites	Leaf			Fruit			Root		
	A	M	C	A	M	C	A	M	C
Alkaloids	+	+	+	+	+	+	+	+	+
Carbohydrates	-	+	-	-	-	-	-	-	-
Glycosides	-	+	-	-	-	-	-	-	-
Flavonoid	+	+	-	+	+	-	-	-	-
Trepenoid	+	+	-	+	+	-	-	-	-
Tannins	+	+	+	-	-	-	-	+	-
Saponin	+	+	+	+	+	+	+	+	+
Protein	-	+	-	-	-	-	-	-	-
Anthroquinone	-	+	-	-	+	-	-	-	-

A – Aqueous; M – Methanol extract; C – Chloroform extract; '+' – Present; '-' – Absent

organism. The methanol extract of plant inhibit the growth of bacteria more than aqueous and chloroform extracts of plants. This trend to show that their active ingredients of plant parts are better extracted with methanol than other solvents. Since, methanol has high polarity; it could dissolve both polar and non-polar compounds in it (Natheer *et al*., 2012).

All the plant extracts showed antibacterial activity against both Gram positive and Gram negative organisms and this was conformity with earlier findings (Pratheeba *et al*., 2013; Abbas *et al*., 2014). Generally, plant extracts are usually more active against Gram positive bacteria than Gram negative bacteria (Basri and Fan, 2005). This may be due to the important characteristic of plant extracts and their compounds is their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exits of critical molecules and ions led to death (Rastogi and Mehrotra, 2002; Gosh *et al*., 2008; de Britto *et al*., 2011). In the present study, MIC values of *Solanum trilobatum* leaf, fruit and root against *Staphylococcus aureus* and *Klebsiella pneumoniae* auspiciously determines that plant parts can be used in treatment of infectious disease (Kunchai and Kitpipit, 2005; Usha *et al*., 2010). A phytochemical compound from plant material is depending on the type of solvent used in the extraction method. The solubility of the active constituents in solution showed some degree of antibacterial activity (Romero *et al*., 2005). It was remarkable that abundance of phytochemicals such as trepenoid, alkaloid, flavonoid, saponin, anthroquinone and tannin in *Solanum trilobatum* constitutes the main antibacterial principle as suggested by many workers (Tambekar and Khante, 2010; Thambiraj and Paulsamy, 2011; Sahu *et al*., 2013; Priya and Chellaram, 2014).

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of novel structures with better safety and efficacy profiles which have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contributions towards human health.

CONCLUSION

The results of present investigation clearly show that the aqueous, methanol and chloroform extracts have inhibitory activity against the tested microorganisms. The inhibition of the growth of these organisms *in vitro* by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The medicinal uses of these plants to heal diseases including infectious one has been extensively applied by people.

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