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In vitro Interactions Between *Solanum torvum* Extracts and Microbes

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Abstract: Plant extracts were traditionally used as medicine without scientific investigations and the outcome was mostly unpredictable. Nutritional substances and secondary metabolites form the basis for their pharmacological actions. *Solanum torvum* is a plant growing in many parts of the world. Their fruits are edible, can withstand heavy drought and with good nutritional content is investigated in this study for its medicinal purposes. The plant's extract from root, fruit and leaf were prepared using different solvents *viz.,* methanol, petroleum ether, chloroform and water. As extractions using different solvents will fetch away maximum phytochemicals from the plant. The interaction between plant extracts and various microbes were studied using cup diffusion method on nutrient agar medium. Among the tests performed methanolic extract of leaf and fruit showed significant antimicrobial activity against most of the test pathogens. This shows that this plant can be used for drug discovery studies as well as clinical use of the plant extract against pathogens like *Bacillus subtilis, Escherichia coli* and *Aspergillus niger*.

Key words: Pharmacological actions · *Solanum torvum* · Antimicrobial activity · Cup diffusion method · Drug discovery

INTRODUCTION

The microbes are increasingly becoming drug resistant [1] and the demand for new drugs to combat resistant strains is also ever mounting up. Plants are inexhaustible source of thousands of phytochemicals and they exhibit actions like antimicrobial, nutritional and pharmacological on human. Secondary metabolites like flavonoids, alkaloids, phenols and terpenes are produced by the plants to protect them from predators and microbes, are used by us as antibiotics [2-4]. These secondary metabolites usually accumulate in the central vacuole of guard cells, epidermal cells of leaves and shoots in compartments in varying concentrations form 5-40% by dry weight [5-10]. It is observed that the concentration of phytochemicals will increase after an initial damage by a pathogen or insect [11-13]. Nitrogen compounds like alkaloids, cyanogenic glycosides, terpenoids [14] and phenolics are some of the chemical defences used by plants. Phenolics are usually not present in bacteria and fungi and algae. Though a few classes of phenolics are recorded from these microbes, flavonoids are completely absent. Flavonoids inhibit spore germination of plant pathogens, especially fungi that affect man [15]. In general fruits contain more nutritional substances and leaves and other parts of plants contain a lot of substances with more medicinal value [16, 17]. Several mechanisms have been observed in phytochemical interactions with microbes. Tannins are toxic to filamentous fungi, yeast and bacteria [18]. Alkaloids like morphine, colchicine, ergoline, strygnine, nichotine and quinine block the cell division by preventing microtubule function [19]. Binding to the nucleic acids and inhibiting DNA repair mechanisms, affecting cell membrane and collapsing cytoskeletal structures, Alkaloids are capable of killing the microbes [20]. Also Alkaloids can inhibit or activate enzymes or alter carbohydrate or fat storage through altering phospodiester bonds. Methanolic extract of *Solanum torvum* fruit, leaf and root contains high concentrations of alkaloids, exerting significant antimicrobial activity. Tannins are also present in medium and high

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concentrations in methanolic and chloroform leaf extracts of this plant acting synergistically with alkaloids in executing the microbes. Hence *Solanlum torvum*, a tall shrub, leaves sinulate, flowers white and berry globose, easily available in india and in many other parts of the world is chosen to be investigated for significant antimicrobial activity with nine microbes. Objective of the study was to identify which plant extract is having the maximum inhibitory property.

MATERIALS AND METHODS

Plant Material: The disease free healthy source of selected plants were collected from the wild areas around Hosur, Tamilnadu, India.

Preparation of Extracts: Plant parts *viz.,* root, leaves and fruits were washed with running water for five times and once in sterile water. Wiped with sterile cotton balls and the plant parts were shade dried for thirty days. Then they were powdered and 50 gms of powder separately from leaf, root and fruit was used for extraction with methanol, petroleum ether and chloroform using soxhlet apparatus [21]. Another 50 gm from each root, fruit and leaf was taken and all were separately boiled with distilled water for four hours and filtered with muslin cloth, centrifuged at 4000 rpm for 15 minutes and supernatant was stored for the aqueous extract of the plant. Then all the extracts were stored in separate glass bottles at 4°C.

Preparation of Culture for Growth: *Escherichia coli* (MTCC 443), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Salmonella typhi* (MTCC 733), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Candida albicans* (MTCC 227), *Penicillium sp* (MTCC 161) and *Fusarium sp* (MTCC 284) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial test cultures were maintained on Nutrient agar slopes and the fungal cultures on SDA slopes. These cultures served as test pathogens for the assay.

Antimicrobial Tests

Antibacterial Activity Tests: The antibacterial activity of the various solvent extracts and the aqueous extracts was determined by cup diffusion method [22] on nutrient agar medium. Cups were made in nutrient agar plates swabbed with the test bacterial suspension using a sterile cork borer (5mm). Then 50µl of all (root, fruit, leaf and aqueous) extracts in various concentrations (25, 50 and 100mg/ml) were added to the cups. The treatments also included 50µl of sterilized distilled water and the solvents separately as control. Antibiotic Gentamycin of Hi-Media Laboratories was also treated for comparison. Zones of inhibition were examined after 24 hours and were measured in millimetres.

Antifungal Activity Tests: The inocula for the assay were prepared from freshly sub cultured 3-4 days old SDA slants. The cultures were scraped with 0.85% saline and transferred to 5ml of distilled water after the deposition of hyphen fragments at the bottom. 1-2 drops of Tween 80 was added and the suspension was vortexed for about 5 min and the density of the suspension was determined using a haemocytometer. The cup diffusion method was used to test the fungal cultures 100µl of fungal inoculum used on SDA plates [23]. Tioconazole was treated for comparison. Zones of inhibition were examined and recorded after 48hours.

Phytochemical Analysis: Phytochemical analysis of petroleum ether, chloroform, methanol and water extracts to detect the presence/absence of metabolites such as steroidal alkaloids, glycosides, saponins, steroids, flavonoids, tannins, cyanidines and anthraquinones were also carried out.

RESULTS

Different solvent and aqueous extracts of leaves, fruits and roots tested in different concentrations against selected bacterial and fungal cultures are listed in Table 1 and 2. Among the four solvent extracts (viz., petroleum ether, chloroform, methanol and aqueous) tested the methanol extract of leaves showed significant antimicrobial activity followed by fruit extract and with minimum activity in the root extract analyses. Comparatively, among the bacterial cultures tested *Bacillus subtilis* was found to be highly susceptible to the methanol extract while *Proteus mirabilis* was less susceptible. The extract exhibited similar activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. Among the fungi, *Fusarium sp., Candida albicans* and *Aspergillus niger* showed significant susceptibility in 50 and 100mg/ml concentrations. *Aspergillus flavus* was found to be less susceptible while no zone formation was obtained with *Penicillium sp.* The other solvent extracts did not show significant antibacterial activity.

Table 1: Antibacterial activity of different extracts of *Solanum torvum*

		25 mg/ml			50 mg/ml			100 mg/ml			
											Ref. compound
Bacteria	Solvents	F	L	R	F	L	R	F	L	R	Gentamycin (1µg/ml)
Bacillus subtilis	C	-	-	-	-	-	-	+	+	-	+++
	M	+	++	-	+	++	-	++	+++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	+	+	+	
Escherichia coli	C	-	-	-	-	-	-	+	+	-	+++
	M	+	++	-	++	++	-	+++	+++	-	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	+	+	-	++	++	-	
Proteus mirabilis	C	-	-	-	-	-	-	-	-	-	++
	M	-	-	-	++	++	-	++	++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	+	+	+	+	+	+	
Pseudomonas aeruginosa	C	-	-	-	-	-	-	-	-	-	+++
	M	-	-	-	++	++	-	++	++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	+	+	+	
Salmonella typhi	C	-	-	-	-	-	-	-	-	-	++
	M	-	-	-	+	++	-	+	++	-	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	+	+	+	
Staphylococcus aureus	C	-	-	-	-	-	-	-	-	-	+++
	M	-	-	-	++	++	-	++	++	-	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	+	+	+	

F, L and R, Fruit, Leaf and Root extracts respectively; C, M, PE and W, Chloroform, Methanol, Petroleum ether and Water (aqueous) extracts respectively; Diameter of zone of inhibition (mm): +, 10-15; ++, 16-20; +++, >20; -, no inhibition.

Table 2: Antifungal activity of different extracts of *Solanum torvum*

		25 mg/ml			50 mg/ml			100 mg/ml			
											Ref. compound
Fungi	Solvents	F	L	R	F	L	R	F	L	R	Tioconazole (10mg/ml)
Aspergillus flavus	C	-	-	-	-	-	-	-	+	-	+++
	M	-	-	-	+	++	-	+	++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	+	+	+	
Aspergillus niger	C	-	-	-	-	-	-	+	+	-	+++
	M	-	-	-	++	++	-	+++	+++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	+	+	-	++	++	-	
Candida albicans	C	-	-	-	-	-	-	-	-	-	+++
	M	-	-	-	++	++	-	+++	+++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	+	+	+	+	+	+	
Fusarium sp.	C	-	-	-	-	-	-	-	-	-	+++
	M	-	-	-	++	++	-	+++	+++	+	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	+	-	+	+	+	
Penicillium sp.	C	-	-	-	-	-	-	-	-	-	+++
	M	-	-	-	-	-	-	-	-	-	
	PE	-	-	-	-	-	-	-	-	-	
	W	-	-	-	-	-	-	-	-	-	

F, L and R, Fruit, Leaf and Root extracts respectively; C, M, PE and W, Chloroform, Methanol, Petroleum ether and Water (aqueous) extracts respectively; Diameter of zone of inhibition (mm): +, 10-15; ++, 16-20; +++, >20; -, no inhibition.

Table 3: Phytochemical screening of different *Solanum torvum* extracts

		Fruit				Leaf				Root			
S.No.	Phytochemicals	C	PE	M	W	C	PE	M	W	C	PE	M	W
1	Alkaloids	++	+	+++	+	++	+	+++	+	++	+	+++	+
2	Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
3	Saponins	++	-	++	-	-	-	-	-	++	-	++	-
4	Steroids	++	+	+++	++	++	+	+++	++	++	+	+++	+
5	Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-
6	Tannins	-	-	-	-	++	+	+++	++	-	-	-	-
7	Cyanidines	-	-	-	-	-	-	-	-	-	-	-	-
8	Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-

-, not detectable, +, low concentration, ++, medium concentration, +++, high concentration

Aqueous extract recorded significant antibacterial activity against *Escherichia coli* and with less significant activity against other test pathogens.

The phytochemical analysis of all extracts (Table 3) revealed that alkaloids and steroids are generally present in both the chloroform and methanol extracts of leaves, roots and fruits of *Solanum torvum*. Saponins were present in chloroform and methanol extracts of root and fruit whereas tannins were present only in methanolic extract of leaf. Other secondary metabolites such as glycosides, flavonoids, cyanides and anthroquinones were absent in all the extracts.

DISCUSSION

Antimicrobial agents are substances of natural, semi-synthetic or synthetic origin that kill or damage the pathogenic microbes, but causing little or no host damage. Throughout centuries these have played an important role in treating a lot of infections. But the development of resistance by the microbes due to high genetic variability has enabled them to rapidly evade the action of antibiotics. This has created major clinical problems in the treatment of infectious diseases [24, 25] and has necessitated the discovery of new drugs with improved actions at an ever increasing rate. It is now known that plants possess thousands of secondary metabolites which can be identified, isolated and used as such or in modified forms as medicines. Plant based antimicrobials have less side effects also. The potency of phytochemicals to check the microbial growth at such low concentration is achieved by the synergetic action of different phytochemicals present in a single plant extract.

India is a mega diversity nation, rich in medicinal plants that have been used from time immemorial as remedies for various infections. The present investigation reveals the antimicrobial activity of this plant and suggests that this plant could be exploited in the management of diseases caused by microbes in human system.

It was found that the solvent extracts showed good antimicrobial activity in comparison with the aqueous extract. The methanol extract of the leaves was found to be efficient against the bacterial pathogens *viz., E. coli* that is frequently associated with Urinary tract infection, a common problem in stressed human being and office bearers who share common toilets, *Pseudomonas aeruginosa* frequently associated with infant bacteria and against the typhoid causing *Salmonella typhi.* The extract was also found to be efficient against the pathogenic fungi *Candida albicans* that causative of Candidiasis and against *Aspergillus niger* the causative of Aspergillosis. The phytochemical profile of the extracts has also been recorded. Since secondary metabolites from natural resources have been elaborated within living systems, they are often perceived as showing more “drug – likeness and biological friendliness than totally synthetic molecules” [26], the therapeutic active components of the *Solanum torvum* methanol extract of leaves and fruits may serve as good candidates for drug development.

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