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Full Length Article

Antibacterial and Antifungal Activity of *Solanum torvum* (Solanaceae)

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ABSTRACT

Leaves, stem, roots and inflorescence of *Solanum torvum* Sw. were extracted in two different organic solvents (chloroform & methanol). Antibacterial and antifungal effects of the extracts were tested on fifteen (six Gram positive & nine Gram negative) human pathogenic bacteria and on eight pathogenic fungi. Methanolic extracts of roots of *S. torvum* exhibited promising antibacterial and antifungal effects on all organisms tested in comparison with that observed in the leaves, stems and inflorescence extracts. The toxicity of the extracts was in the following order; root>stem>inflorescence>leaf. The minimum inhibitory concentration (MIC) values of methanolic extract of roots of *S. torvum* were in the range between 64-128 µg mL^{-1}. Chloroform extracts of roots were more toxic (LC_{50} 35.4629 ppm) than other extracts analyzed in Brine shrimp test. In conclusion, *S. torvum* appears to be an attractive material for the development of antimicrobial drugs and environment friendly biopesticides.

Key Words: *Solanum torvum*; Antibacterial; Antifungal activity; Extract; Leaf; Stem; Root; Inflorescence; Solvent

INTRODUCTION

Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to very much promising. Relevant literature showed that ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann & Hamburger, 1991) and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally.

Solanum torvum is a prickly, tomentose, erect shrub, 1.5-3 m high, leaves having no prickles, white bell-shaped flowers and lobed fruits seated on the calyx belonging to the family Solanaceae. It is a common plant found throughout the Indian subcontinent. In Bangladesh it is common in dry regions and often occurs gregariously. It is locally known as tit begoon, gota begoon or hat begoon in Bengali and commonly known as turkey berry, susumber, gully-bean thai eggplant or devil's fig. Common people of Bangladesh especially the tribes use the fruit of *S. torvum* as vegetables in their daily diet (Ghani, 1998).

Leaves have been reported to contain the steroidal gluco-alkaloid, solasonine. In addition, they contain steroidal sapogenins, neochlorogenin, neosolaspigein and solaspigenine. They have also been found to contain triacontanol, tetratriacontanic acid, z-tritriacontanone, sitosterol, stigmasterol and campesterol. Fruits also contain the gluco-alkaloid, solasonine, sterolin (sitesterol-D-glucoside), protein, fat and minerals (Yuanyuan *et al*., 2009).

Different parts of the plants are used as sedative, diuretic and digestive. They are also used in the treatment of coughs and colds (Yuanyuan *et al*., 2009). Leaves are used as haemostatic. Extract of the fruits and leaves are said to be useful in case of liver and spleen enlargement and in the treatment of cough. Paste of root is used to cure cracks in feet. The fume of burning seeds is inhaled for toothache (Bhakuni *et al*., 1962 & 1969). Due to the notable medicinal value of *S. torvum,* it was considered of interest to carry out a phytochemical and antimicrobial investigation of this species and the results leading to the antimicrobial screening are presented in this paper (Belboukhari *et al*., 2002; Belboukhari & Cheriti, 2002, 2006).

MATERIALS AND METHODS

Extract preparation: The whole plant of *Solanum torvum* was collected in October 2004 from the Botanical Garden, University of Rajshahi, Bangladesh. The botanical identification was completed by Prof. A.T.M. Nadiruzzaman, Department of Botany, University of Rajshahi and a voucher specimen (No. M.A. Bari 36,

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collection date 16.10.2004) is deposited to the Department of Botany, University of Rajshahi, Bangladesh. Leaves, stems, roots and inflorescence of *S. torvum* were separated and dried at room temperature for 10-14 days. The dried plant parts were powdered in a hand grinding machine. The sufficient amount (250 g) of dust of each plant parts was extracted in two different organic solvents *viz.* chloroform ($CHCl_3$) and methanol (MeOH) using Soxhlet's apparatus. The extracts were dried in a vacuum rotary evaporator at 40ºC under reduced pressure and were subjected to various chromatographic analyses (Vacuum liquid chromatography, Preparative TLC. etc.)

Microorganism and medium: The microorganisms used in the present study were six Gram positive (*Staphylococcus aureus, Bacillus cereus, B. megaterium, B. subtilis, Sarcina lutea* & *Streptococcus-β-haemolyticus*) & nine Gram negative (*Salmonella typhi, Shigella dysenteriae, S. shiga, S. sonnei, S. boydii, Escherichia coli, Klebsiella sp., Pseudomonua aeruginosa* & *Proteus sp*.) human pathogenic bacteria as well as eight pathogenic fungi (*Aspergillus fumigatus, A. niger, A. flavus, Vasin factum, Mucor* sp., *Candida albicans, Fusarium oxysporum* & *Colletotrichum falcatum*). These microorganisms were collected from the Department of Microbiology, Microbiological Laboratory, Institute of Nutrition and Food Science (INFS), University of Dhaka, International Centre for Diarrhoea Diseases Research Bangladesh (ICDDRB), Dhaka. All microorganisms were derived from clinical isolates. The fungi were grown in Potato Dextrose agar (PDA) and/or Nutrient agar media, whereas the bacteria were grown on DIFCO agar medium (Alexopolos & Bebeke, 1962).

Antimicrobial screening: Sterile 5 mm diameter blank disc were used to impregnate of the methanol extracts. Discs were stored at -5ºC prior to use. Tests were performed using the standard disc diffusion assay method (Chabner *et al*., 1996). Sample discs were prepared by dissolving 1 mg and 4 mg of each crude extracts (Leaf, stem, root & inflorescence) in 200 µL of solvents (Chloroform & Methanol) to obtain a concentration of 50 and 200 µg 10 μL^{-1}. Extract impregnated discs were placed on agar and incubated either at 37^0C for 24 h to 48 h for bacteria or at 25^0C for 24 h for fungi. Antibacterial and antifungal effects were determined by measuring the diameter with a transparent scale in millimeter based on appearance of the clear zones of inhibition on the discs. The results were compared with control antibiotic and antifungal drugs (Bauer *et al*., 1966; Barry, 1976). The antibiotic drug is Ciprofloxacin at 30 µg/disc, whereas antifungal drug is Nystatin at 50 µg $disc^{-1}$.

Brine shrimp test: Toxicity was studied using the larvae of brine shrimp nauplii, *Artemia salina* L (Meyer *et al*., 1982; McLaughlin, 1991a). For each sample, 0.32 mg of crude extracts were initially dissolved in 100 µL of pure dimethyl sulfoxide (DMSO) to make the extracts hydrophilic. Then 1.9 mL of distilled water was added to get a concentration of 320 µg 2 mL^{-1} for each extract and was used as a stock solution. Sample extract solutions of 160, 80, 40, 20 and 10 ppm were made from the stock solution using by a serial tube dilution technique (Reiner, 1982) and were placed in five different vials. Ten nauplii were then placed in each vials. The concentration of DMSO in these vials was not allowed to exceed 50 µL 5 mL^{-1} of brine, because above this concentration cytotoxicity due to DMSO may arise. In the control vials the same volume of DMSO and 5 mL of sea water were taken. After 24 h of incubation, the vials were examined and the number of survivors in each vials were counted. The experiments were replicated thrice along with a standard, ampicillin trihydrate.

The mortality was corrected using Abbott's formula (Abbott, 1925):

$$p_t = \frac{p_o - p_c}{100 - p_c} \times 100$$

Where P_t = Corrected mortality

P_o = Observed mortality

P_c = Control mortality.

The observed data was the subject to Probit analysis according to Finney (1974) and Busvine (1971).

RESULTS AND DISCUSSION

Antibacterial activity: Results showed that the highest zone of inhibition was prominent for the control (Ciprofloxacin). Most of the Gram positive bacteria showed clear zone of growth of inhibition except *S. lutea* at 50 µg $disc^{-1}$ of crude extracts of root. Similarly, at 200 µg/disc showed clear zone of growth of inhibition in root. Out of nine Gram negative bacteria, only *S. typhi* and *Sh. dysenteriae* shows clear zone of inhibition at both concentrations of chloroform and methanolic crude extracts of root. No zone of inhibition was recorded of the rest seven Gram negative bacteria. The crude extracts of both solvents, the root exhibited bigger and more prominent clear zone at 200 µg $disc^{-1}$ against *Streptococcus-β-haemolyticus* (Table I). Chehregani *et al*. (2007) reported that some species of *Allium* had antibacterial activity against some tested bacteria. El-Adley *et al*. (2007) studied antibacterial effects of low power laser light and volatile oil of Fennel (*Foeniculum vulgare* var. dulce) on Gram-positive and Gram-negative bacteria and found remarkable antibacterial activity against tested plants, where the diameter of inhibition zone ranging from (6-20 mm). The present findings are similar to those of Chehregani *et al*. (2007) and El-Adley *et al*. (2007).

Minimum inhibitory concentration: The minimum inhibitory concentration (MIC) of the methanolic crude extracts of roots of *S. torvum* were determined against *B. cereus, B. subtilis, Streptococcus-β-haemolyticus, S. typhi* and *Sh. dysenteriae* using by a serial tube dilution technique (Reiner, 1982) and the results are presented in Table II. The results indicated that root extract of *S. torvum* had prosperity

Table I: Effects of leaf-, stem-, root -and inflorescence extracts of *Solanum torvum* and standard ciprofloxacin on growth of Gram$^+$ and Gram$^-$ bacteria

Test Organisms	Diameter of zone of inhibition (mm)																
	Chloroform extract								Methanol extract								Ciprofloxacin 30 (µg/disc)
µg/disc $^{-1}$ →	Leaf		Stem		Root		Inflor*		Leaf		Stem		Root		Inflor*		
	50	200	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive bacteria																	
Staphylococcus aureus	-	-	07	09	07	13	07	12	-	-	07	19	07	22	07	21	34
Bacillus cereus	-	-	-	09	06	14	07	13	-	-	-	19	08	22	-	21	33
B. megaterium	-	-	-	-	07	12	-	-	-	-	-	-	08	19	-	-	35
B. subtilis	-	-	-	-	07	18	-	-	-	-	-	-	08	21	-	-	34
Sarcina lutea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33
Streptococcus.-β-haemolyticus	-	-	-	-	09	21	-	-	-	-	-	-	09	24	-	-	34
Gram negative bacteria																	
Salmonella typhi	-	11	07	14	07	19	07	13	-	16	07	20	07	21	07	21	35
Shigella dysenteriae	-	-	-	-	07	18	-	17	-	-	-	-	07	20	-	18	34
S. shiga	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
S. sonnei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33
S. boydii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35
Klebsiella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Pseudomonus aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35
Proteus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34

Notes: Inflor* = Inflorescence,- = Not active

Table II: Minimum inhibitory concentrations (MIC) of methanolic root extract of *S. torvum* against five pathogenic bacteria. Each test tube contained 1 mL of either NB or PDB medium

Test tube No.	Nutrient broth or potato dextrose broth medium added (mL)	Diluted solution of chloroform extract from root(µg mL^{-1})	Inoculums added (µL)	Bacillus cereus	Bacillus subtilis	Streptococcus-β-haemolyticus	Salmonella typhi	Shigella dysenteriae
1	1	512	10	-	-	-	-	-
2	1	256	10	-	-	-	-	-
3	1	128	10	-	-	-	-	-
4	1	64	10	+	-	-	-	+
5	1	32	10	+	+	+	+	+
6	1	16	10	+	+	+	+	+
7	1	8	10	+	+	+	+	+
8	1	4	10	+	+	+	+	+
9	1	2	10	+	+	+	+	+
10	1	1	10	+	+	+	+	+
Cm	1	0	0	-	-	-	-	-
Cs	1	1024	0	-	-	-	-	-
Ci	1	0	10	+	+	+	+	+
Results of MIC values (µg mL^{-1})				128	64	64	64	128

Notes: +=Indicates growth, - = Indicates no growth

to inhibit bacterial growth even at low concentration (64-128 µg mL^{-1}). The MIC values are same in both the cases against Gram positive and Gram negative bacteria. The lowest value was detected for *B. subtilis* and *Streptococcus-β-haemolyticus* but highest in *B. cereus* in case of Gram-positive bacteria. *S. typhi* showed the lowest activity as compared to *Sh. dysenteriae*. *B. cereus* and *Sh. dysenteriae* showed the excellent antibacterial activity. The results indicated that the plants have MICs between 64-128 µg mL^{-1} as relatively good antibacterial agents. Similar results were found by Akinpelu and Onakoya (2006) in some medicinal plants used in folklore remedies in South-Western and Chehregani *et al*. (2007) in some *Allium* species from Hamedan-Iran.

Antifungal activity: No zone of inhibition was observed when exposed to leaf-and inflorescence extracts but a prominent zone of inhibition was observed when exposed to stem extracts of 200 µg disc^{-1} in both the solvents. Root extracts derived from either methanol or chloroform inhibited growth of *Vasin factum, A. fumigatus* and *C. albicans* at both 50 and 200 µg/disc. But at 200 µg disc^{-1} the root extract of *S. torvum* resulted in bigger and more prominent zone of inhibition of the growth of three fungi (Table III) but no zone of inhibition was observed in *F. oxysporum* and *C. falcatum* in both the solvents as well as in both the extracts and concentrations also.

Brine shrimp lethality bioassay: The mortality rates of brine shrimps nauplii increased with the increase in the concentration of the tested crude extracts. The LC_{50} values of the crude extracts of leaf, inflorescence, stem and root and the ampicillin trihydrate were determined using Probit analysis (McLaughlin *et al*., 1991b) and were found to 124.29, 119.14, 92.25, 35.46 and 16.18 ppm for chloroform and 497.54, 453.18, 325.71, 203.59 and 16.18 ppm for

Table III: Effects of extracts of *Solanum torvum* and the drug nystatin on fungal growth *in vitro* (leaf, stem and inflorescence

Test Organisms	Diameter of zone of inhibition (in mm)																
	Chloroform extract								Methanol extract								Nystatin 50 (µg/disc)
µg/disc $^{-1}$ →	Leaf		Stem		Root		Inflor*		Leaf		Stem		Root		Inflor*		
	50	200	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
Aspergillus fumigatus	-	-	-	09	07	10	-	-	-	-	-	12	07	13	-	-	28
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29
A. flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
Vasin factum	-	-	-	-	09	13	-	-	-	-	-	-	11	15	-	-	28
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
Candida albicans	-	-	-	08	07	11	-	-	-	-	07	11	09	13	-	-	29
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
Colletotrichum falcatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29

Notes: Inflor* = Inflorescence, - = Not active

Table IV: Effects of ampicillin trihydrate, leaf-, stem-, root - and inflorescence extract (lethality bioassay) on survival of brine shrimp nauplii

Solvents	Extracts	LC_{50} (ppm)	95 % Confidence limit (ppm)		Regression equation	χ^2 Values
			Lower	Upper		
	Ampicillin	16.18	7.17	36.53	Y = 4.06 + .78 X	0.04
	Leaf	124.29	62.77	246.10	Y = 2.80 + 1.05 X	0.46
Chloroform	Stem	92.25	52.55	161.94	Y = 2.86 + 1.09 X	1.19
	Root	35.46	23.64	53.21	Y = 3.13 + 1.21 X	0.55
	Inflorescence	119.14	56.78	250.02	Y = 3.04 + 0.95 X	0.26
	Ampicillin	16.18	7.17	36.53	Y = 4.06 + 0.78 X	0.04
	Leaf	497.54	72.76	3402.35	Y = 3.03 + 0.73 X	0.13
Methanol	Stem	325.71	73.69	1439.61	Y = 3.03 + 0.78 X	I .19
	Root	203.59	46.03	900.58	Y = 3.58 + 0.61 X	0.30
	Inflorescence	453.18	67.46	3044.46	Y = 3.1279 + 0.70 X	0.19

methanol, respectively (Table IV).

In the Brine Shrimp Test all of the crude extracts were found to be lethal to brine nauplii indicating that the extracts are biologically active. Table IV also indicates that the root extracts of chloroform and methanol are more active (35.46 ppm & 203.59 ppm, respectively) than those derived from leaf, stem and inflorescence.

CONCLUSION

The present investigation revealed that the chloroform and methanol extracts of root of *S. torvum* are highly active against *Streptococcus-β-haemolyticus* and *Vasin factum,* respectively. The MIC results indicate that the methanol extract of root has prosperity to inhibit bacterial growth even at low concentrations (64-128 µg mL^{-1}). The chloroform extracts of root are more toxic (LC_{50} 35.4629 ppm in the BST test) than the other extracts tested. Further studies on this plant are essential for (a) identification and isolation of the bioactive compounds in the extracts and their mechanism of action and (b) determination of the pharmacological and toxicological effects of the extracts exhibiting inhibition of microbial growth.

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