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Evaluation of antimicrobial potential of different extracts of *Solanum xanthocarpum* Schrad. and Wendl.

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Antimicrobial activity of the aqueous and organic solvent extracts of different parts (roots, stems, leaves and fruits) of *Solanum xanthocarpum* Schrad. and Wendl. against Gram-positive and Gram-negative bacteria and a fungus was evaluated. Plant extracts of *S. xanthocarpum* were prepared in distilled water and in organic solvents, *viz*. ethanol, benzene, acetone and methanol. Agar well diffusion technique was used to assess the antimicrobial activity of various extracts against Gram-positive (*Staphylococcus aureus*, *S. epidermidis*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and the fungus *Aspergillus niger.* The diameter of zone of inhibition was taken as an indicator of antimicrobial effect. Except aqueous extracts of different parts of *S. xanthocarpum* , extracts prepared in organic solvents showed antimicrobial activity against the test organisms. A strong inhibition of *P. aeruginosa* was caused by the ethanolic and methanolic extracts of *S. xanthocarpum.* Thus, *S. xanthocarpum* could be considered as a potential source of natural antimicrobials.

Key words: *Solanum xanthocarpum*, antimicrobial activity, organic solvents.

INTRODUCTION

Over the last few decades, a great interest has developed in searching for antimicrobial drugs from natural plant products. This interest primarily arises from the belief that drugs derived from plants are safe and dependable compared with synthetic drugs that may have adverse effects on host besides their high cost. Natural antimicrobials came from a wide array of sources including plants, animals and microorganisms (Gordon and David, 2001). Researchers have so far discovered approximately over 10,000 biologically active compounds of microbial origin (Shahidi et al., 2004). Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases (Davis, 1994; Service, 1995; Shears, 2000). Therefore, there is an exigency to discover new and efficient antimicrobials from other sources such as plants (Cordell, 2000; Karaman et al., 2003; Raghavendra et al., 2005).

Solanum xanthocarpum has profound use in Ayurveda and folklore medicine. It is a commonly growing perennial herbaceous weed with bright green leaves and zig-zag stem, mostly found in the arid region. It is supposed that the plant has solasonine in its different parts, which is responsible for its medicinal value (Oudhia, 2007). In the present study an attempt was made to screen different extracts prepared from various parts of *S. xanthocarpum* for its antimicrobial action against Gram-positive and Gram-negative bacteria and fungi.

MATERIALS AND METHODS

Plant material

Different parts of *S. xanthocarpum* Schrad. and Wendl., including root, stem, leaves and flowers, were collected during December 2005 from a locality in Sirsa (Haryana). The plant was brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water. Various parts of the plant were separated accordingly and dried in shade and then grinded. The dried powders of different parts were stored separately in airtight containers prior to extraction.

Microorganisms

Strains, including fungi and bacteria were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. Two Gram-positive strains, *Staphylococcus*

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aureus (MTCC 87) and *Staphylococcus epidermidis* (MTCC 435), two Gram-negative strains, *Escherichia coli* (MTCC 41) and *Pseudomonas aeruginosa* (MTCC 424), and one fungus, *Aspergilus niger* (MTCC 404), were used in the present investigation as test organisms.

Culture media and chemicals

The culture media, Nutrient Agar (NA), Nutrient Broth (NB) and Czapek Yeast Extract Agar (CYA) used for the growth of microorganisms, were purchased from Hi- Media. All other chemicals, including organic solvents used for the extraction of the plant metabolites, were of analytical grade.

Preparation of plant extracts

For preparation of ethanol and benzene extracts, 10 g of each of the dried and powdered materials were extracted separately with 100 ml of ethanol and benzene for 48 h at room temperature, followed by filtration through several layers of muslin cloth. Extracts were concentrated under reduced pressure. The concentrated products were kept at 4^{o}C prior to test.

For the preparation of acetone and methanol extracts, 10 g of the dried and powdered materials were extracted separately with 100 ml of benzene for 48 h at room temperature. After filtration, the residue was further extracted with 100 ml of chloroform at room temperature for a further 48 h. The extracts were filtered through muslin cloth and the resulting residue was extracted with 100 ml of acetone for 48 h at room temperature, followed by filtration. One fraction of the filtrate was stored as acetone extract at 4ºC in a refrigerator until used and the other fraction of the acetone extract was then extracted with 50 ml methanol at room temperature, followed by filtration through muslin cloth. The filtrate was stored at 4ºC before testing for its antimicrobial activity. For the preparation of aqueous extracts, 10 g of plant material was extracted with 100ml of distilled water. The mixture was heated slowly at 40ºC for 6 h in an oven and filtered through several layers of muslin cloth. The filtrate was concentrated to 1/5 of the original volume by evaporation in shaded conditions and stored at 4ºC until used.

Antimicrobial activity

Antimicrobial activity of the above mentioned extracts was determined, using the agar well diffusion assay method (Perez et al., 1990) . Approximately 20 ml of molten and cooled media (NA and CYA) were poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms (*S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*) were grown in nutrient broth for 24 h. A 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. For *A. niger*, a spore suspension of the fungus was prepared in 2 ml sterilized distilled water in a test tube and 100 µl of spore suspension was spread on CYA plates. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Five wells (four on the periphery and one in the center) were prepared in the agar plates. The wells in each plate were loaded with 100 µl of various plant extracts, *viz*. root, stem, leaf and fruit. The central well in each plate was used as a control and loaded with 100 µl of solvent or sterilized distilled water as the case may be. Various antibiotics and fungicides (Table 2) were used as positive controls. The plates containing the bacteria and extracts were incubated at 37^{o}C (*for E. coli*, *S. epidermidis* and *P. aeruginosa*) and at 30^{o}C (for *S. aureus and A. niger*). All the tests were repeated in triplicates.

The antimicrobial activity was taken on the basis of diameter of zone of inhibition, which was measured at cross-angles after 24 h of incubation and the mean of three readings is presented in Table 1. Percent inhibition of bacterial/fungal microorganisms was calculated after subtracting the value of control (solvents) from the value of extracts using the control as standard.

RESULTS AND DISCUSSION

The antimicrobial activities of various extracts of different plant parts of *S. xanthocarpum* are presented in Table 1. All the plant extracts tested, exhibited different degrees of antimicrobial activity against the tested microorganisms. The aqueous extracts of various parts of *S. xanthocarpum* did not show any antimicrobial activity, except leaf and fruit extracts against *P. aeruginosa* and *A. niger*. It is clear from Table 1 that most of the extracts were effective against the fungus *A. niger*.

The ethanolic extract of leaf of *S. xanthocarpum* was found to be effective against all tested microorganisms with the inhibition zone ranging from 13.8 to 25.5 mm. It was also observed that leaf and fruit extracts were more effective as compared to root or stem extracts of the plant. No activity was observed in root and stem extracts against *S. epidermidis*.

Benzene extracts of different parts of *S. xanthocarpum* exhibited antimicrobial activity against the test organisms. However, *E. coli* was quite resistant to all the extracts prepared in benzene. The extracts obtained from leaf and root of *S. xanthocarpum* showed an antimicrobial activity, having an inhibition zone of 17 and 17.5 mm against *A. niger* and *S. epidermidis*, respectively.

The extracts prepared in acetone were effective against most test microorganisms, except *P. aeruginosa*. The acetone extracts of leaf and stem parts had an antimicrobial effect showing an inhibition zone of 23.3 and 16 mm against *A. niger* and *E. coli*, respectively. However, *E. coli* was found to be resistant to leaf and fruit extracts. The methanol extracts of different parts of *S. xanthocarpum* showed antimicrobial activity against all the test microorganisms with the inhibition zone ranging from 7.3 to 26.3 mm in root and leaf extracts against *P. aeruginosa* and *A. niger*, respectively.

When the results of the present investigation were compared with standard antimicrobial drugs (Table 2), it was observed that the antimicrobial effect of ethanol and methanol extracts of leaves and fruits of *S. xanthocarpum* were acceptable with respect to the standard antibiotics.

From these results it was observed that with the solvents ethanol and methanol, active antimicrobial compounds could be extracted from various plant parts. A variety of solvents have been used but, except for phenolics bound to insoluble carbohydrates or proteins, majority of the compounds could be extracted with methanol or acetone (Van Sumere, 1989). It is also clear from the present study that the Gram-negative bacterium *E. coli* was comparatively more resistant to the extracts.

However, *S. aureus*, the common wound pathogen, was sensitive to most of the extracts. Antibacterial activity

Table 1. *In vitro* antimicrobial activity (zone of inhibition in mm) of different plant extracts of *Solanum xanthocarpum*.

Extract	Organism	Control	Root	Stem	Leaf	Fruit
Ethanol	*Staphylococcus aureus*	11.0	14.1 (28)	13.3(20)	13.8 (25)	25.8 (134)
	Staphylococcus epidermidis	11.0	-	-	18.6 (69)	17.8 (61)
	Escherchia coli	12.0	7.8 (-35)	7.2 (-4)	16.3 (35)	10.8 (-10)
	Pseudomonas aeruginosa	10.3	13.5 (31)	24.1 (133)	15.8 (53)	12.8 (24)
	Aspergillus niger	7.6	20 (163)	12.5(64)	25.5 (235)	20.8 (22)
Benzene	*Staphylococcus aureus*	6.2	10.5 (69)	11.5(85)	12.3 (98)	10.0 (61)
	Staphylococcus epidermidis	3.5	17.5 (400)	14.5 (314)	13.1 (274)	11.1 (217)
	Escherichia coli	19.0	-	10.8 (-43)	13.8 (-27)	-
	Pseudomonas aeruginosa	-	10.8	11.0	11.0	-
	Aspergillus niger	10.0	13.5	14.1(41)	17.0 (70)	13.0 (30)
Acetone	*Staphylococcus aureus*	7.0	11.6 (66)	7.3(4)	8.5 (21)	6.2 (-11)
	Staphylococcus epidermidis	7.3	10.0 (37)	11.5(58)	11.3 (55)	9.3 (27)
	Escherchia coli	11.0	14.5 (32)	16.0(45)	-	-
	Pseudomonas aeruginosa	-	-	-	-	-
	Aspergillus niger	12.0	14.6 (22)	14.2(18)	23.3 (94)	14.5 (21)
Methanol	*Staphylococcus aureus*	11.0	14.1 (28)	13.4(22)	15.3 (39)	12.3 (11)
	Staphylococcus epidermidis	8.8	11.5 (31)	14.5(64)	12.5 (42)	12.6 (43)
	Escherchia coli	10.6	11.4 (7)	9.4 (-11)	9.9 (-6)	11.9 (12)
	Pseudomonas aeruginosa	11.1	7.3 (-34)	12.6(14)	11.6 (4)	14.6 (32)
	Aspergillus niger	10.8	19.5 (81)	13.6(26)	26.3 (144)	24.6 (128)
Aqueous	*Staphylococcus aureus*	-	-	-	-	-
	Staphylococcus epidermidis	-	-	-	-	-
	Escherchia coli	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	12.8
	Aspergillus niger	-	-	-	10.0	16.5

- = No activity, All values are mean of three replicates.
The values in parenthesis show percent increase or decrease (minus values) over the control excluding the diameter of agar wells.

Table 2. Antimicrobial activities of various antibiotics used as positive controls.

Antibiotics	Diameter of zone of inhibition (mm)			
	Escherchia coli	***Staphylococcus aureus***	***S. epidermidis***	***Aspergillus niger***
Streptomycin	16 ± 0.1	15 ± 0.2	13 ± 0.7	ND
Penicillin	18 ± 0.4	17 ± 0.3	15 ± 0.2	ND
Ampicillin	17 ± 0.2	16 ± 0.1	16 ± 0.4	ND
Tetracycline	20 ± 0.1	22 ± 0.2	17 ± 0.3	ND
Fluconazole	ND	ND	ND	14 ± 0.2
Clotrimazole	ND	ND	ND	16 ± 0.3
Ketoconazole	ND	ND	ND	13 ± 0.2

ND = Not Determined, ± = Standard Deviation
All values are mean of three replicates.

of *Aloe vera* leaf gel extracts against *S. aureus* has been evaluated by Pawar et al. (2005). They observed a complete inhibition of *S. aureus* at 50% leaf gel concentration. There are meager reports on the antimicrobial activity of *S. xanthocarpum* extracts in the literature. Similarly, Javanmardi et al. (2003) reported that the total phenolic content of different accessions of *Ocimum* are in the range of 6.07 to 65.5 mg GAE per dry weight. The total phenolics of alcoholic extracts may function as strong antimicrobial compound(s) against the tested microorganisms. Further work on isolation of various plant metabolites is required to ascertain the antimicrobial potential of *S. xanthocarpum*.

It is evident from the present study that the extracts of

examined parts of *S. xanthocarpum* were active against the tested microorganisms. Based these findings and the medicinal potential of this plant, we suggest that further phytochemical studies be performed to determine the major active principles responsible for the antimicrobial effect of this plant. The results support the use of this plant in folklore medicine for treatment of infectious diseases.

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