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Screening of antibacterial potential of root extracts of *Solanum xanthocarpum* Schrad. & Wendl

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Ten pathogenic bacteria were tested against the crude organic extracts (ethanol, hexane, chloroform and ethyl acetate) of roots of *Solanum xanthocarpum* Schrad. & Wendl = *Solanum surattense* Burm. F. Extracts were active against Gram-positive bacteria *Staphylococcus aureus, Enterococcus faecalis* and Gram-negative bacteria *Erwinia chrysanthemi*, *Klebsiella pneumoniae*, *Pasteurella multocida*. The hexane extract showed maximum inhibition against the test microorganisms (ZOI, 16 to 21 mm) followed by ethanol, methanol and chloroform extract (ZOI, 6 to 17 mm). The inhibitory activity of these extracts was found very effective as compared to ampicillin (10 µg) and erythromycin (15 µg) standard antibiotics which were used as positive controls against these tested microorganisms.

Key words: *Solanum xanthocarpum*, Solanaceae, antibacterial activity, disc diffusion method.

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan et al., 2006). The biological diversity in the Indian Himalayan Region (IHR) especially in Kumaun Himalaya has been a source of medicine for millions in the country and elsewhere (Tripathi, 2007). The diversity of medicinal plants in the region is also manifested by the presence of the 31% native, 15.5% endemic and 14% threatened plants of total Red Data Book plant species of Indian Himalaya region (Dhar et al., 2000). Various plants being the effective source of both traditional and modern medicines are genuinely useful for primary health care. Ethnobotanists and natural drug pharmacists have reported that a number of medicinal plants are capable of curing various diseases (Dhar et al., 1968; Basu et al., 2002). The genus Solanum (family Solanaceae) is a vast genus of herbs, shrubs and rarely trees with about 1500 species widely distributed in the temperate and tropical regions of the world. Fifty species have been reported in India and out of these 12 species are found in Central Himalayan region of Uttarakhand viz: *Solanum anguivi*, *S. erianthum*, *S. hispidum*, *S. incanum*, *S. melongena*, *S. nigrum*, *S. pseudocapsicum*, *S. sacforthianum*, *Solanum surattense*, *Solanum torvum, Solanum tuberosum* and *Solanum viarum* (Gaur, 1999; Gupta, 1968). Few ornamental exotics have been introduced into the gardens. This genus is economically very important as several species are sources of food, fodder and drugs (Dasture et al., 1977).

S. xanthocarpum Schrad and Wendl = *S. surattense* Burn. F. grows wild in the central Himalayan areas of Uttarakhand, India with an elevation of 1,200 to 1,800 m (Starchy, 1974). Its fruits are used as medicine in fever, spas-modic cough, asthma and gonorrhea and floral buds in eye ailments. Leaves and fruit extract sometimes used to intoxicate fishes and fruits garlanded around the neck of infants to rid off jaundice (Gaur, 1999). The dried whole plant showed significant improvement in some respiratory diseases like bronchial asthma (Vadnera et al., 2008; Khare et al., 1995). Its roots are one of the con-stituents of well known Ayurvedic preparation “Dasmula Ashva” (Amir and Kumar, 2004). The ethanolic extract of

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S. xanthocarpum leaves was found to be effective against *Escherichia coli* (12 mm), *Staphylococcus aureus* (11 mm) and methanolic extract of roots showed that the maximum zone of inhibition (13 to 20 mm) against *A. niger* (Salar, and Suchitra, 2009).

The literature survey showed few reports of antimicrobial activity of root extracts of *S. xanthocarpum*. A detailed study of the antibacterial potential of different solvent extracts of *S. xanthocarpum* roots on several gram-positive and -negative microorganisms of clinical importance has hence be carried out.

MATERIALS AND METHODS

Plant materials

The roots of the plant were used for the extraction and were collected from the river bank of Koshi, Ramnager district Nainital (Uttarakhand) at an elevation of 1,200 m in the month of January when the plant was in its flowering stage. The plant was identified at the Department of Botany, R. H. Govt. P. G. College Kashipur where a herbarium specimen was deposited for further reference.

Extraction procedure

The roots of the plant were thoroughly washed and dried under shade at room temperature (20±2°C). The dried material was powdered in a grinder. Finely powdered roots were subjected to serial extraction with ethanol, hexane, chloroform and ethyl acetate. Each mixture was stirred every 24 h using sterile glass rod. At the end of extraction, each extract was passed through Whatman's filter paper No. 1 (Whatman, UK). The filtrate was concentrated with a rotary evaporator under vacuum at 35°C and was utilized for the experiments.

Antimicrobial assay

The test organisms used in this study were laboratory isolates of the pure culture of Gram-positive *Bacillus subtilis* (MTCC 441), S. aureus (MTCC 737), *Enterococcus faecalis* (MTCC 439), and Gram-negative *Agrobacterium tumefaciens* (MTCC 609), *Erwinia chrysanthemi* (KUMSCC 328), *Xanthomonas phaseoli* (KUMSCC 327), *E. coli* (MTCC 1687), *Proteus vulgaris* (MTCC 1771), *Klebsiella pneumoniae* (MTCC 109) and *Pasteurella multocida* (MTCC 1148). Standard pure identified cultures of these test bacteria were procured from the Institute of Microbial Technology, Chandigarh, India and Plant Pathology Department, G.B. Pant University of Agriculture and Technology, Pantnagar, India as microbial type culture collection, and maintained in the laboratory by regular sub-culturing on to nutrient agar. The isolates were separately cultured on nutrient agar plates for 24 h. 20 ml of the medium was poured in to sterile Petri plate (80 mm diameter) and allowed to solidify. A colony of each test organism was sub cultured on 10 ml nutrient broth and incubated at 37 ± 1°C for 8 h. 1 ml of the broth culture was then used to flood the agar plates.

Antibacterial testing

The preliminary screening of antibacterial activity was conducted at 1000 µg/ml concentration by disc-diffusion method (Bauer et al., 1966; Cruickshank, 1968), Streptomycin 30 µg/disc (Himedia) and erythromycin 15 µg/disc (Himedia) were used as positive controls, while respective solvent was used as negative control on all the bacteria to compare the zone of inhibition with that of the extract sample. The MIC values were determined at five different concentrations (500, 250, 125, 62.5, and 31.25 µg/ml) of the extract and each set of experiment was done in triplicate.

The nutrient agar having pH value of 7.3 ± 0.2 was used as a medium which was autoclaved at 121°C for 30 min at 15 lb pressure. Whatman's filter paper No. 1 was used to prepare discs with 5 mm diameter. Microorganisms were streaked in radial pattern on the agar plate (Afolayan et al., 2003) by rotating the plate at approximately 60° each time to ensure an even distribution of innoculum. Once plates were inoculated with the microorganism, four filter discs of 5 mm diameter each containing the root extract, solvent as the negative control and two containing the standard antibiotics (positive control) were applied in clockwise pattern with the help of forceps. The plates were inverted and placed in an incubator set to 37±1°C. After 24 h of incubation, each plate was examined for growth inhibition. The diameter of complete inhibition zone was measured to the nearest whole mm.

RESULTS AND DISCUSSION

The antibacterial testing of the ethanol root extract of *S. xanthocarpum* showed active inhibition against all tested bacterial strains. *S. aureus, E. coli, B. subtilis, K. pneumoniae* and *P. multocida* were found sensitive against all the tested extracts whereas *E. chrysanthemi* and *X. phaseoli* were found to be the most resistant among all the tested bacterial strains.

Maximum growth inhibition against *S. aureus* was shown by ethanol extract (22 mm) at 1000 µg/ml which was followed by ethyl acetate (21 mm), hexane (19 mm) and chloroform extract (15 mm) while ethyl acetate extract showed maximum growth inhibition against *P. multocida* (21 mm) followed by ethanol (20 mm), chloroform (15 mm) and hexane extracts (14 mm) (Figure 1).

Ethyl acetate extract showed maximum inhibitory effect (19 mm) against *E. chrysanthemi* followed by chloroform and ethanol extract (17 mm), respectively, and hexane extract (16 mm), while the ethanol extract showed maximum zone of inhibition (18 mm) against *K. pneumoniae* followed by hexane (16 mm), chloroform (10 mm) and ethyl acetate extracts (14 mm). The zone of inhibition values are summarized in Table 1 and Figure 1. For determination of MIC value, those pathogens were taken against the extract which showed significant inhibition (>10 mm) in the initial screening.

S. aureus showed MIC value at lowest concentration (62.5 µg/ml) against ethanol and ethyl acetate extract followed by hexane and chloroform extract at (125 µg/ml). *E. faecalis* and *K. pneumoniae* showed inhibition at lowest concentration (62.5 µg/ml) against ethanol extract (Table 2). All the bacterial strains were found more sensitive to the tested extracts in comparison to commercially available susceptibility discs of erythromycin and streptomycin used as positive controls.

Similarly, Javanmardi et al. (2003) reported that the total phenolic content of different accessions of the genus *Ocimum* are in the range of 6.07 to 65.5 mg GAE per dry

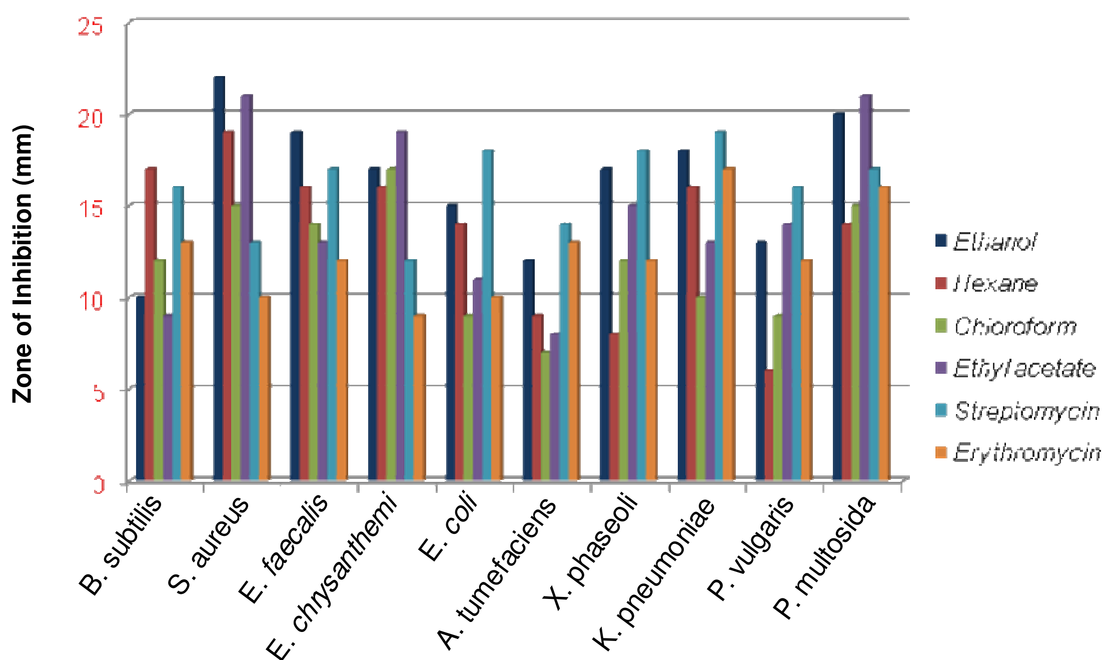

Figure 1. Antibacterial activity of different solvent extracts of *S. xanthocarpum.*

Table 1. Antibacterial activity of different extracts of *S. xanthocarpum*.

Micro-organism (bacteria)	Zone of Inhibition (mm) (1000 µg/ml)					
	Ethanol	Hexane	Chloroform	Ethyl acetate	Streptomycin	Erythromycin
B. subtilis	10	17	12	09	16	13
S. aureus	22	19	15	21	13	10
E. faecalis	19	16	14	13	17	12
E. chrysanthemi	17	16	17	19	12	09
E. coli	15	14	09	11	18	10
A. tumefaciens	12	09	07	08	14	13
X. phaseoli	17	08	12	15	18	12
K. pneumoniae	18	16	10	13	19	17
P. vulgaris	13	06	09	14	16	12
P. multocida	20	14	15	21	17	16

weight. The total phenolics of alcoholic extracts may function as strong antimicrobial compound(s) against the tested microorganisms.

Relying upon the results obtained for its inhibitory effect on various microorganisms tested indicates that the different extracts of *S. xanthocarpum* root part could be useful for treating diseases of plants and animals. Therefore, study on the extracts of this plant can be recommended for the preparation of effective antimicrobials.

The study on the broad-spectrum antibacterial activity of this plant justifies its known uses in dysentery and antiseptic agents in traditional medicine.

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Table 2. Minimum inhibitory concentration values of different extracts of root of *Solanum xanthocarpum.*

Microorganism (bacteria)	Concentrations of different solvent extracts (µg/ml)																			
	Ethanol					Hexane					Chloroform					Ethyl acetate				
	500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25
B. subtilis	08	07*	-	-	-	12	08	07*	-	-	09*	-	-	-	-	-	-	-	-	-
S. aureus	13	09	08	07*	-	11	08	07*	-	-	10	07	06*	-	-	12	09	08	07*	
E. faecalis	12	08	07	07*		09	07*	-	-	-	08	06*	-	-	-	09	06*	-	-	-
E. chrysanthemi	10	08	07*	-	-	08	07*	-	-	-	11	09	06*	-	-	10	08	07*	-	-
E. coli	10	08*	-	-	-	07	07*	-	-	-	-	-	-	-	-	08	06*	-	-	-
A. tumefaciens	08	06*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
X. phaseoli	10	08	06*	-	-	-	-	-	-	-	07*	-	-	-	-	11	08	06*	-	-
K. pneumoniae	11	09	08	08*	-	10	08	07*	-	-	06*	-	-	-	-	08	06*	-	-	-
P. vulgaris	09	07*	-	-	-	-	-	-	-	-	-	-	-	-	-	10	07	06*	-	-
P. multocida	12	09	07*	-	-	09	07*	-	-	-	09	07	06*	-	-	11	08	07*	-	-

*MIC: Minimum inhibitory concentration.

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