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ANTIMICROBIAL ACTIVITY OF A FEW MEDICINAL PLANTS AGAINST GRAM NEGATIVE BACTERIA

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ABSTRACT : The methanol and aqueous extracts of leaves of five different medicinal plants, *Solanum nigrum* L., *Solanum torvum* Sw., *Solanum trilobatum* L., *Solanum surattense* Burm. and *Solanum melongena* L. belonging to Solanaceae family were used for the investigation of antibacterial studies. In antibacterial screening performed by disc diffusion method against two gram negative bacteria namely *Xanthomonas campestris* (plant pathogen) and *Aeromonas hydrophila* (animal pathogen), it was found that the methanol extracts of all the plant samples showed significant activity against the two tested bacteria. The methanol extracts of *S. nigrum, S. torvum* and *S. surattense* exhibited clear zone of inhibition against the tested micro organisms. Among these three samples, the MIC value of *S. surattense*, determined by serial dilution technique, was found to be 32µg/ml and 64µg/ml against *Xanthomonas campestris* and *Aeromonas hydrophila* respectively.

Key words: MIC, antibacterial activity, gram negative bacteria and plant extracts.

INTRODUCTION

Xanthomonas is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. The hosts of this genus include atleast 124 monocotyledonous and 268 dicotyledonous plants, among which the rice bacterial blight, cabbage black rot disease, and citrus blight disease are the most serious diseases, which cause a big economic impact on agricultural production every year. Chemical control has been proved efficient and economical in controlling blight disease. However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (Cuthbertson and Murchie 2005; Singh 2003). Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (Mandavia *et al*, 1999). This seriously hinders the management of diseases of crops and agriculture products. Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of pathogenic microorganisms (Mahajan and Das, 2003).

Aeromonas hydrophila is one of the causative agents for diarrhoeal infections in children and immunocompromised patients. These are ubiquitous water borne organisms and have gained importance as human and animal pathogens causing gasterointestinal and extraintestinal infections (Agger *et al,* 1985, Ananthan and Alavandi 1999, Vila *et al*, 2003).

The genus *Solanum* L. consists of over 2000 species distributed worldwide is the largest in Solanaceae and is one of the largest among all flowering plants (Olmstead & Palmer, 1997). The species are medicinal herbs (Caicedo & Schaal, 2004) and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments (diabetes, cholera, bronchitis, high blood pressure) and as laxatives (Daunay & Chadha, 2004). *S. nigrum ,S.torvum, S. trilobatum ,S. surattense* and *S. melongena* are important medicinal plants.

The antibacterial studies of the above medicinal plants were already investigated against some common human pathogenic bacteria (Anushia *et al,* 2009). The present study analysed the antibacterial activities of the selected five plants against *Xanthomonas campestris* (plant pathogenic bacteria) and *Aeromonas hydrophila* (animal pathogenic bacteria).

MATERIALS AND METHODS

Collection of plant materials

Fresh plants were collected randomly from the region of Tirunelveli, India. The plants together with their medicinal uses and common names are given in Table 1. Fresh plant material was washed; shade dried and then powdered using the blender and stored in air tight bottles.

Table 1: Medicinal plant species selected for antibacterial activity

Plant species	Local name in Tamil	Part used as medicine	Medicinal uses
Solanum nigrum	Manathakkali	Whole plant	fever and allay pain
Solanum torvum	Sundaikkai	Leaves and fruits	leucoderma
Solanum trilobatum	Thuthuvalai	Leaves	cough and asthma
Solanum surattense	Kandankathari	Whole plant	blood pressure
Solanum melongena	Katharikkai	Leaves and fruits	Blood purifier

Extraction of plant materials

Aqueous extraction

10 g of plant powder was added to 100 ml of distilled water and mixed well. After 24 hours the supernatant collected and concentrated to make the crude extract. It was stored at 4^0C (Harbone JB, 1973).

Methanol extraction

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4^0C (Harbone JB, 1973).

Bacterial strains

Aeromonas hydrophila (MTCC No. 646), *Xanthomonas campestris* (MTCC No. 2286) were procured from the Institute of Microbial Technology (IMTECH), India and were used to examine the antibacterial activity. The microorganisms were maintained at 4^0C on nutrient agar slants.

Antibacterial assay

The antibacterial activity assay was performed by agar disc diffusion method (Bauer *et al*., 1966). Muller Hinton agar medium was seeded with 100µl of inoculum (1×10^8 CFU/ml). The impregnated discs containing the test sample (100µg/ml) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Kanamycin 30µg/disc, Neomycin 10µg/disc) and blank discs (impregnated with solvent and water) were used as positive and negative control. The plates were then incubated at 37^0C C for 24 h to allow maximum growth of the microorganisms (Bauer *et al*., 1966). The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and mean of the three experiments was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude methanol *S. nigrum, S. torvum* and *S. surattense* against *X.campestris* and *A.hydrophila* were determined by using serial dilution technique (Reiner, 1982). 1 mg/ml of the sample solutions of all the extracts were prepared using Dimethyl Sulfoxide (DMSO). In this technique a large number of test tubes were used and each of the test tubes was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (inoculum contains 1×10^6 cells/ml) followed by incubation at 37^0C for 24 hours to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC). Another three test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth (Reiner, 1982). Experiments were done in triplicate and repeated twice.

Statistical analysis

All data were expressed as mean ± SD. Statistical analyses were evaluated by one-way ANOVA followed by Tukey HSD test. Values with $P< 0.005$ were considered statistically significant.

RESULTS AND DISCUSSION

Antibacterial activity assay

Aqueous extract

Antibacterial activity of aqueous extracts of all the five plants are presented in Table 2. Highly significant antibacterial activity was observed in *S. surattense* followed *S. nigrum* and *S. torvum,* respectively against two tested pathogens. Among the two pathogens *A.hydrophila* was highly susceptible.

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Table 2: Antibacterial activity of leaves extracts of selected medicinal plants

Plant samples	Extracts (100µg/ml)	*Xanthomonas campestris* (inhibition zone in mm)	*Aeromonas hydrophila* (inhibition zone in mm)
Solanum nigrum	Aqueous	9.00±1.00	10.00±0.00
	Methanol	15.00±1.00	12.00±0.82
Solanum torvum	Aqueous	10.33±0.57	11.33±1.15
	Methanol	12.66±1.52	14.66±0.57
Solanum trilobatum	Aqueous	10.00±1.00	11.00±1.00
	Methanol	11.33±0.57	14.33±0.57
Solanum surattense	Aqueous	15.33±0.57	16.00±0.00
	Methanol	19.00±1.00	18.00±0.82
Solanum melongena	Aqueous	5.00±0.00	10.00±0.00
	Methanol	7.33±0.47	6.33±0.47
Kanamycin(30µg/ml)	Antibiotic	15.00±0.85	12.66±0.47
Neomycin (10µg/ml)	Antibiotic	16.33±0.47	15.66±0.47
Control aqueous	Blank	0.00±0.00	0.00±0.00
Control methanol	Blank	0.00±0.00	0.00±0.00

Data given are mean of three replicates ± standard error, $p < 0.005$

Solvent extract

The ANOVA analysis of the data revealed that among the six plants *S. surattense* ($p<0.005$) showed highly significant activity against the tested pathogens (Table 2). Tukey HSD analysis of the data revealed that *X.campestris* was highly susceptible. Antibacterial activity of methanol and aqueous extract of *S. nigrum* and *S. torvum* was highly significant when compared to Kanamycin and Neomycin

Minimum Inhibitory Concentration (MIC)

The MIC of *S. torvum* was 128µg/ml against *X. campestris* and *A. hydrophila*. Then the MIC values of *S. surattense* were 32µg/ml and 64µg/ml against the above two microorganisms. Similarly the MIC values of *S. nigrum* were 64µg/ml and 128µg/ml against *X.campestris* and *A. hydrophila* respectively. Hence it is concluded that the extracts of *S. surattense, S. nigrum* and *S. torvum,* showed inhibition of bacterial growth even at low concentrations (Table 3). Among these three plants, the MIC value of *A.lanata* is the lowest against both *X.campestris* and *A. hydrophila*. Hence *S. surattense* shows significant ($p<0.005$) bactericidal activity compared to other plants. According to the results of antibacterial assay, the methanol extracts of *S. surattense* and *S. nigrum* might be used as antibacterial agents against *X.campestris* and *A.hydrophila* which affect plants and animals respectively.

Table 3: MIC Values of three plant extracts (µg/ml) against two bacteria

Name of bacteria	*Solanum torvum*	*Solanum surattense*	*Solanum nigrum*
X.campestris	128.00±0.00	32.00±0.00	64.00±0.00
A. hydrophila	128.00±0.00	64.00±0.00	128.00±0.00

Results are mean from three sets of experiments, each set in triplicate ± SD, $p < 0.005$

Ghosh *et al.*, (2008) evaluated the antibacterial potentiality of hot aqueous and methanol solvent extracts of mature leaves of *Polyalthia longifolia* against six reference bacteria. Highest antibacterial activity was observed against *K. pneumoniae* in both the extracts followed by *E.coli* in hot aqueous extract and *B. subtilis* in methanol extract as evident from MIC values. Shirsat (2008) reported the anti – phytopathogenic activity of crude and methanol extract of leaves, stem bark, seed and dry fruit of *Terminalia thorelli*, against four phyto pathogens. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Rastogi and Mehrotra, 2002). The results of the present investigation is successful in identifying the antibacterial activity of selected medicinal plants which will help in further identifying the nature of the bioactive principle and its solubility, isolation and characterization of the active principle responsible for the activity.

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