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AZADIRACTA INDICA A JUSS. - A POTENTIAL ANTIMICROBIAL AGENT AGAINST *XANTHOMONAS CAMPESTRIS*

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ABSTRACT : The leaves, stem, flowers and fruits of *Azadiracta indica* .A Juss. which have some medicinal applications were investigated. Phytochemical analysis gave positive results for steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins. The crude methanol extracts showed growth inhibitory effects on *Xanthomonas campestris*. The methanol extract of the leaves and fruits showed significant inhibitory effect when compared with positive controls, neomycin and kanamycin respectively. The stem and flowers extracts show marked antibacterial activity. Among these samples, the MIC value of leaves and fruits determined by serial dilution technique was found to be 32µg/ml and 64µg/ml against *Xanthomonas campestris* respectively.

Key words: Phytochemical analysis, crude extracts, Antibacterial screening.

INTRODUCTION

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish 2008). Ayurveda regarded neem (*Azadirachta indica* Family: Meliaceae) as a cure for many ailments, predominantly due to its superb antimicrobial activity. Almost every part of the tree is bitter and finds application in indigenous medicine. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activity (Kirtikar and Basu 1987). Neem tree is evergreen tree found in most tropical countries. Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children (Chattopadhyay *et al.,* 1993). Flavonoids, flavonoglycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem. The biological activities and medicinal properties of neem have recently been reported (Venugobal *et al.,* 1994).

Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (Mandavia *et al*., 1999). *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 (Singh et al., 2003). Chemical control has been proved efficient and economical in controlling plant 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 (Singh *et al.,* 2003; Cuthbertson and Murchie, 2005). Natural plants derived compounds contribute a lot in fight against pathogens (Vyvyan, 2002). Various plant extracts have also been examined for their antibacterial activity with the objective of exploring environmentally safe alternatives of plant disease control. Thus with the objective to contribute to these studies, the antibacterial activity of methanol extract of different parts of *Azadirachta indica* was investigated against *Xanthomonas campestris*.

MATERIALS AND METHODS

Collection of plant materials

Fresh plant and plant parts were collected randomly from the region of Tirunelveli, India. Fresh plant material was washed; shade dried and then powdered using the blender and stored in air tight bottles.

Methanol extraction

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 42 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4^0C (Harbone, 1973).

Phytochemical analysis

Phytochemical analysis of methanol extracts of different parts of *A. indica* was conducted following the procedure of Brindha *et al.,* (1981).

Antibacterial assay

Xanthomonas campestris (MTCC No. 2286) was procured from the Institute of Microbial Technology (IMTECH), India. The antibacterial activity of methanol extracts of different parts of *A. indica* was tested in disc diffusion method following the procedure of 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) were used as positive and negative control. The plates were then incubated at 37^0C 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 extracts of leaves, stem, flowers and fruits of *A.indica* 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 $P< 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis of the leaves, stem, flowers and fruits of *A.indica* showed the presence of steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins (Table 1).

Table 1: Phytochemical analysis of methanol extracts of selected plant parts

Compounds	Leaves	Stem	Flowers	Fruits
Steroids	+	+	+	+
Triterpinoids	+	+	_	+
Reducing sugars	+	+	+	+
Sugars	+	_	+	+
Alkaloids	+	+	+	+
Phenolic compounds	+	+	+	+
Flavonoids	+	+	+	+
Catechins	+	_	+	+
Saponins	+	_	_	_
Tannins	+	+	+	+
Anthroquinones	+		_	+
Amino acids	+	+	_	+

Antibacterial assay

From the results of the antimicrobial screening (Table 2), the methanol extracts of leaves have significant antimicrobial activities compared to the other parts of the selected plant with respect to the tested bacteria *X. campestris*. The ANOVA analysis revealed that methanol extracts of leaves showed highly significant inhibitory effect ($p < 0.05$) when compared with neomycin and fruits also showed significant inhibitory effect ($p < 0.05$) when compared with kanamycin which are used as positive controls. The methanol extracts of stem and flowers of the selected plant also show marked inhibitory effects.

Table 2: Antibacterial activity of different parts of selected plant against *Xanthomonas campestris* compared with two positive controls (zone of inhibition in mm)

Samples	Methanol solvent	Neomycin	Kanamycin
Leaves	29.20±0.47	17.00±0.82	8.00±1.60
Stem	15.12±0.12		
Flowers	20.60±0.18		
Fruits	21.05±0.08		

Data given are mean of three replicates ± standard error. $P < 0.05$

Minimum Inhibitory Concentration (MIC)

The MIC of leaves of *A.indica* was 32µg/ml against *X. campestris*. Then the MIC values of stem and fruits were 128µg/ml and 64µg/ml against the tested microorganism respectively. Similarly the MIC value of flowers was 128µg/ml against *X.campestris*. Hence it is concluded that the methanol extracts of all the parts of *A. indica* showed inhibition of bacterial growth even at low concentrations (Table 3).

Table 3: MIC Values of methanol extracts four parts of the selected plant (µg/ml) against the tested bacteria

Parts of Plant	***Xanthomonas campestris***
Leaves	32.00±0.00µg/ml
Stem	128.00±0.00µg/ml
Flowers	64.00±0.00µg/ml
Fruits	128.00±0.00µg/ml

Results are mean from three sets of experiments, each set in triplicate ± SD, $p < 0.05$

Among these four parts, the MIC value of leaves of *A.indica* is the lowest against both *X.campestris*. Hence the leaves of *A. indica* shows significant ($p<0.05$) bactericidal activity compared to other parts of the plants. According to the results of antibacterial assay, the methanol extracts of leaves and fruits of neem plant might be used as antibacterial agents against *X.campestris* which affect plants.

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. Ghosh *et al.*, (2008) evaluated the antibacterial potentiality of hot aqueous and methanol solvent extract of mature leaves of *Polyalthia longifolia* against six reference bacteria. The bactericidal action of different solvent extracts of *Azadirachta indica* were tested *in vitro* against the worth of citrus canker disease causing pathogen, *X. axonopodis* (Manonmani *et al.*, 2009). Here the antibacterial activity of different parts of A. indica was screened against *X. campestris.* 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).

CONCLUSIONS

Hence the present study suggests that the methanol extracts of leaves and fruits of neem may be used as antibacterial agents against phytopathogenic bacteria which cause more dangerous infectious diseases in plants.

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