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Acalypha indica L. - A Potential Antimicrobial Agent against *Erwinia herbicola*

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ABSTRACT

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

INTRODUCTION

Agriculture is critical for human welfare, providing food, employment, income, and assets. In the past, agricultural research and development largely focused on improving production, productivity, and profitability of agricultural enterprises. An important negative effect of agricultural intensification is disease (John and Delia, 2011). Diseases of crop plants caused by bacteria, viruses, and nematodes can be severe, reduce crop yield and quality, and generally are more difficult to control. For example, plant pathogenic bacteria affect so many crops and vegetables such as tomato, spinach, onion, lettuce, celery, carrots, strawberry, cucumbers etc (Stary and Hans, 1998).

Erwinia herbicola is widespread in nature as an epiphyte on many plants. Some strains of this bacteria have evolved into plant pathogens that induce yellow-brown lesions on honeydew melons (*Cucumis melo* L.) fruits, blight on *Aglaonema* spp, wilting of cut rose (*Rosa* spp) flowers, rot of alfalfa (*Medicago sativa* L.) sprouts, internal necrosis of immature cotton (*Gossypium* L.) boll, internal rot to peach (*Prunus persica* L.), apricot (*Prunus armeniaca)*, plum (*Prunus cerasifera*) and apple (*Malus domestica*) fruits and internal discoloration to tomato (*Lycopersicon esculentum* Mill). It is an occasional problem on squash and pumpkins; watermelons are rarely affected (Galal *et al*., 2003). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs (Uniyal *et al.*, 2006). Hence the aim of the present study is to screen the antibacterial activity of methanol extract of different parts such as roots, stem, leaves and flowers of the medicinal plant namely *Acalypha indica* against the plant pathogenic bacteria *Erwinia herbicola.*

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 40^0C (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

Erwinia herbicola (MTCC No. 3609) 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 100 µl 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 30 µg/disc, Neomycin 10 µg/disc) and blank discs (impregnated with solvent) were used as positive and negative control. The plates were then incubated at 37^oC for 24 h to allow maximum growth of the microorganisms. 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 roots 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^oC 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. Experiments were done in triplicate.

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 roots of *A. indica* showed the presence of steroids, triterpenoids, 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	Roots
Steroids	+	+	+	+
Triterpenoids	+	+	-	-
Reducing sugars	+	+	+	+
Sugars	+	+	+	+
Alkaloids	+	+	+	+
Phenolic compounds	+	+	+	+
Flavonoids	+	+	-	+
Catechins	+	+	+	-
Saponins	+	-	-	-
Tannins	+	+	+	-
Anthraquinones	+	+	-	-
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 *E. herbicola*. The ANOVA analysis revealed that methanol extracts of leaves showed highly significant inhibitory effect ($p < 0.05$) when compared with neomycin and stem also showed significant inhibitory effect ($p < 0.05$) when compared with kanamycin which is used as positive controls. The methanol extracts of root and flowers of the selected plant also showed marked inhibitory effects.

Table 2: Antibacterial activity of different parts of selected plant (zone of inhibition in mm)

Samples	Methanol solvent	Kanamycin	Neomycin
Leaves	25.20±0.47	8.00±1.60	17.00±0.82
Stem	21.12±0.12		
Flowers	19.60±0.18		
Roots	18.05±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 32 µg/ml against *E. herbicola.* Then the MIC values of stem and roots were 128 µg/ml and 64 µg/ml against the tested microorganism respectively. Similarly, the MIC value of flowers was 128 µg/ml against *E. herbicola*. 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 of four parts of the selected plant (µg/ml)

Parts of Plant	MIC value
Leaves	32.00±0.00 µg/ml
Stem	64.00±0.00 µg/ml
Flowers	128.00±0.00 µg/ml
Fruits	128.00±0.00 µg/ml

Among these four parts, the MIC value of leaves of *A. indica* is the lowest against both *E. herbicola*. 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 stem of *Acalypha* plant might be used as antibacterial agents against *E. herbicola* 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 phytopathogens. 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 *E. herbicola.* An important characteristic of plant extracts and their components is their hydrophobicity, which enables 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).

CONCLUSION

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

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I humbly present this work to the eternal almighty.

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