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Antibacterial Activity of Methanolic Extract of Medicinal Plant-*Murraya paniculata (Linn.)* against *Xanthomonas citri*

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A B S T R A C T

The present study aimed at evaluating the antibacterial activity of methanolic extract of *Murraya paniculata* (leaves and stem) against *Xanthomonas citri* using agar well diffusion method on different concentration. *Murraya paniculata* commonly known as orange jasmine or honey bush, in Hindi this plant is popular as "Kamini". There are numerous uses of *Murraya paniculata* in traditional medicine for the treatment of different diseases of plants and animals. In India most of the plant pathogenic diseases were caused by bacteria. In the Gwalior-Chambal division of Madhya Pradesh so many plant diseases are caused by different species of *Xanthomonas*. In this research work our main aim is to develop the natural bactericides. In this study some effective results have observed by the use of methanolic extract of *Murraya paniculata*. The results of inhibitory activity of the methanolic plant extract compare with the standard antibiotic Kanamycin. The zone of inhibition of extract of *Murraya paniculata* at the concentration of 200 µg/ml was 25 mm (leaves) and the zone of inhibition of the standard antibiotic Kanamycin was 32 mm. Hence, the result of *Murraya paniculata* (leaves) is so closer to the standard antibiotic. Therefore, there is a scope to use *Murraya paniculata* (leaves) extract against *Xanthomonas citri* as bactericides.

Introduction

Plant pathogenic microorganisms like-bacteria, fungi, viruses cause many serious diseases in plants throughout the world (Vidhyasekaran, 2002). Recently, bacterial pathogens and their control is a serious threat in agricultural practices. Beattie, 2006 has studied that some bacteria are associated with plants are diverse in the habitats they occupy their phylogeny and they affect on plants and environmental health. Different diseases of plants are caused by different genera like – *Pseudomonas, Xanthomonas, Xylella, Xylophillus, Acidovorax, Agrobacterium, Erwinia, Pantoea, Ralstonia, Burkholderia, Clavibacter, Streptomyces, Spiroplasma* and *Phytoplasma* (Ellis *et al*., 2008). Among different genera, *Xanthomonas* is a very important kind of plant pathogenic bacteria, which is one of the main causal organisms in different diseases in crops, fruits and vegetables in all over the world. It is a

Gram- negative bacterial genera. Pathovars of *Xanthomonas* are known to cause disease on several vegetable and crops and are reported to have developed resistance to Kanamycin, Ampicillin, Penicillin and Streptomycin (Weller and Saettler, 1980; Nafade and Verma, 1985; Verma *et al.*, 1989; Rodriguez *et al.*, 1997). *Xanthomonas* species can cause bacterial spots and blights of leaves, stems and fruits, citrus canker on a wide variety of plant species (Boch *et al*., 2010). There have been different methods for control of bacterial diseases in plants by the use of different methods like spraying with antibiotics and copper compounds along with pesticides are usually applied. Microorganisms have developed resistance against antibiotics and this has created risk in the treatment of infectious diseases and phytopathogens (Nagumanthri *et al.*, 2012). Bruce, 2010 has studied that synthetic pesticides have provided cost-effective control of pests over the last few decades but have several disadvantages like damage to environment and also provide direct affect to human health as well. However, the development of new pesticides is important towards combating resistant pathogens. The increasing incidence of pesticides resistance is further fueling the need for new generation of pesticides which are natural and ecofriendly (Kavitha & Satish, 2011). Recently, in plant pathology the great attention is dedicated to search and produce medicinal plant extracts. This is one of the best ways to substitute synthetic pesticides and antibiotics for inhibition of plant pathogens and also provide beneficial effect to environment and living being in the world. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associate with synthetic antimicrobials (Nagumanthri *et al.*, 2012). However, the decade has been witnessed an increase in the investigation of plants as a source of plant and human disease management (Kamaba and Hassan, 2010). In the report of World Health Organization, 2008 medicinal plants that contain biochemical compound can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. *Murraya paniculata* (Linn.), commonly known as orange jasmine or honey bush belongs to the family Rutaceae and is also known as Chalcas exotica, Chalcas paniculata, and Camunium exoticum (Seidemann, 2005). It is distributed throughout the world like- India, Bangladesh, tropical Srilanka to Myanmar, southern China, Taiwan, Thailand and eastwards throughout the Malaysian region to northeastern Australia and Caledonia. *M. paniculata* is commonly used in traditional medicines for the treatment of diarrhea, abdominal pain, stomach ache, dysentery, headache, edema, thrombosis and stasis of blood. Thus, the objective of present study is to determine the antibacterial activity of methanolic extract of leaves and stem of *Murraya paniculata* against *Xanthomonas citri.*

Materials and Methods

Collection of Plant material

Different parts of plants like leaves and stem were collected from Jiwaji University campus, Gwalior (M.P.), India during the month of February to March 2016.

Preparation of Plant Extract

Fresh leaves and stem of *M. paniculata* were washed 2-3 times with tap water and subjected to shade drying at room temperature. The dried plant material was powdered using a clean mixer grinder and filled in air tight container and store in a dry

place on room temperature until analysis (Harborne, 1979).

Methanol Extraction

The powdered materials of *M. paniculata* were extracted with methanol. During extraction the ratio was taken 1:10 placed into Soxhlet apparatus which run for ten cycles. The duration of each cycle was about 55 minutes. After the completion of ten cycles the color of powdered material was disappeared or light. After extraction the crude extract were evaporated at 40^0C with the help of Hot plate stirrer. The extracts were collected and stored at 4^0 C in sterile air tight containers for further analysis (Harborne, 1979).

Antibacterial assay

Xanthomonas citri (ITCC NO. BN0001) was procured from the Indian Agriculture Research Institute (IARI), Pusa, New-Delhi. The antibacterial activity of methanol extract of leaves and stem of *M. puniculata* was tested by agar well diffusion method (Akpata and Akinrimisi, 1977). The extract of leaves and stem of *M. puniculata* were dissolve in DMSO (Dimethyl Sulphoxide) in a concentration of 100mg/ml. In this method wells were made in Muller Hinton Agar (MHA) medium using sterile cork borer after the spreading of bacteria. The method is suitable for organisms that grow rapidly overnight at 35-37^0C. The previously inoculated bacterial strain was spread on MHA. After few minute, five wells were made in each Petri plate and loaded with different concentration (40, 80, 120, 160 and 200µg/ml). Similar concentration (40, 80, 120, 160 and 200 µg/ml) of antibiotic Kanamycin solution was added in another plate for positive control. Plates were incubated at 37^0C for 24hrs. The zone of inhibition of bacterial growth around each well is measured and the susceptibility is determined. Antibacterial activity was evaluated by measuring zone of inhibition by using Hi-media zone scale.

Results and Discussion

The antibacterial activity of Methanolic extract of *M. paniculata* was investigated using agar well diffusion method, against *Xanthomonas citri* at different concentration (40µg/ml, 80µg/ml, 120µg/ml, 160 µg/ml and 200µg/ml) after 24 hours of incubation we observed that the zone of inhibition was shown in Table 1:

Zone of Inhibition (ZOI)

The antibacterial activity of Methanol extract of *M. puniculata* (leaves) showed the maximum zone of inhibition 25mm at the concentration of 200 µg/ml against *X.citri* followed by 22mm at the same concentration of *M. puniculata* (stem), 21mm (stem) at 160 µg/ml, 20mm (leaf) at same concentration, 20 mm(leaf) at 120 µg/ml, 16mm (stem) at same concentration, 13mm (stem) at 80 µg/ml, 12mm at same concentration and 12mm (leaf) at 40 µg/ml, 10mm (stem) at the same concentration of Methanol extract of *M. puniculata*. Highly significant antibacterial activity was observed in *M. puniculata* (leaf) 25mm at the concentration of 200 µg/ml We have done parallel experiment with Positive control antibiotic Kanamycin which shows significant antibacterial activity against *X.citri.,* which are showed in Table -1 and Graph 2.

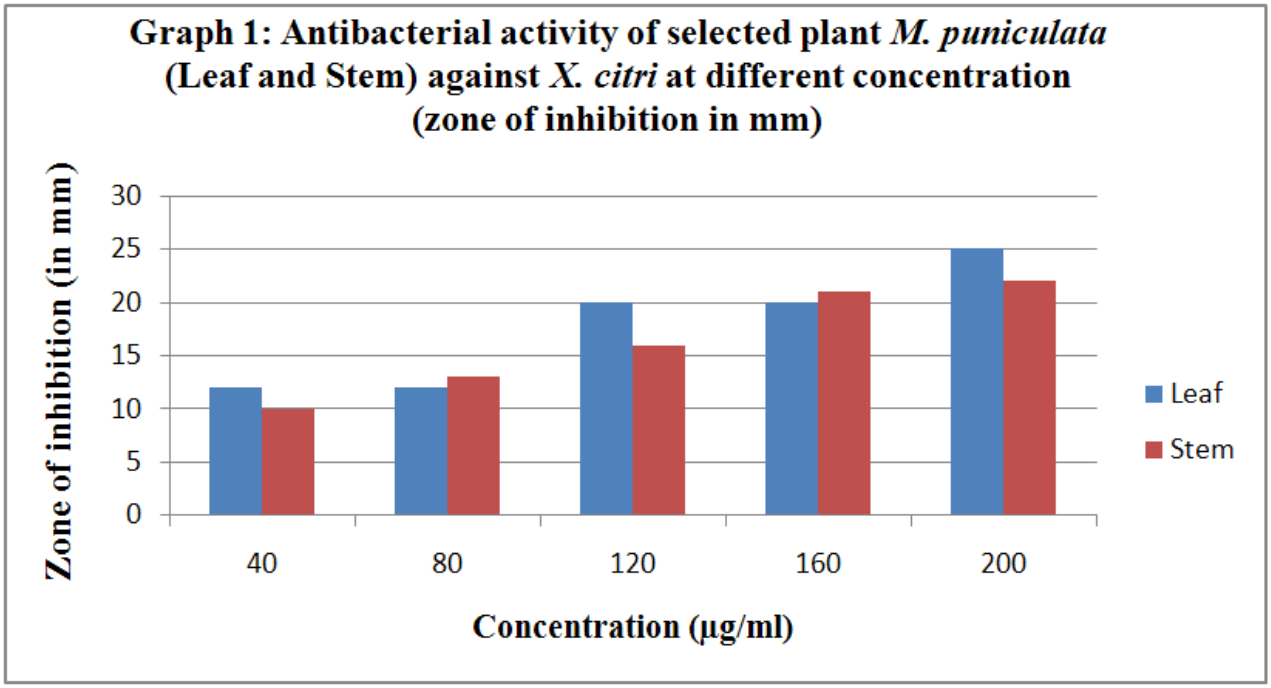
Herbal medicines play an important role to cure various plant and animal diseases. Recently the works on plant pathogen by various scientists have been extensively increased throughout the world (Satish *et al*., 1999; Britto *et al*., 2011). In this study we

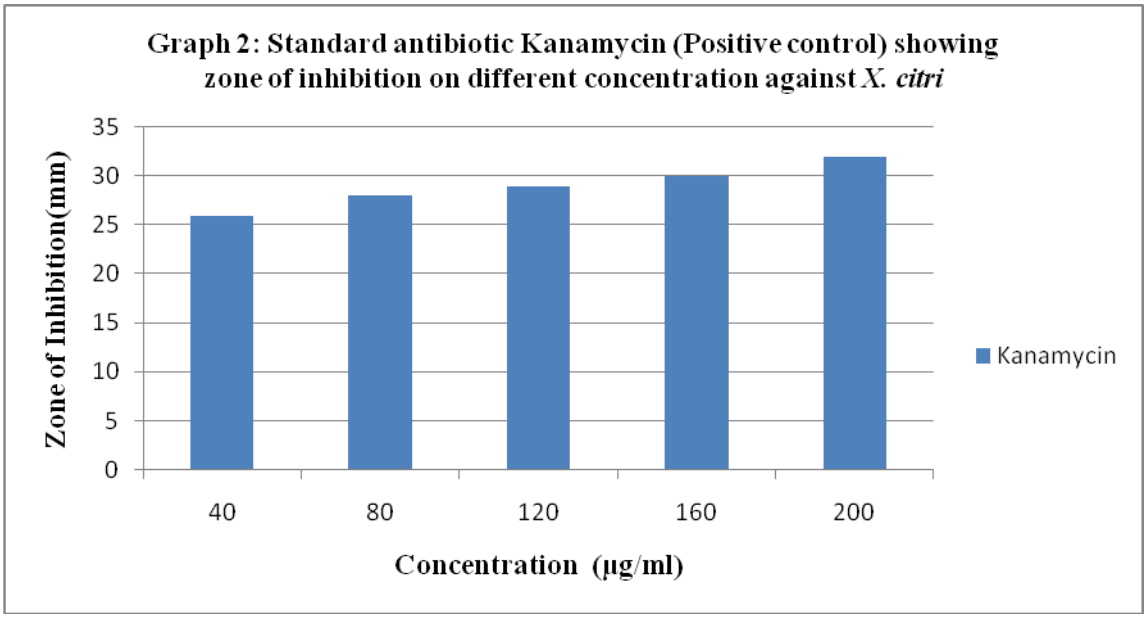
checked the antibacterial activity of methanolic extract of *M. paniculata* against *X.citri*. *X.citri* severely affects many citrus crop and cause big economic impact on farmers throughout the world (Prakash *et al*., 2012).

Table.1 Antibacterial activity of *M. paniculata* (Leaf and Stem) against *Xanthomonas citri* on different concentration compared with positive controls (zone of inhibition in mm)

S. No.	Concentration (µg/ml)	Sample	Methanolic extract (ZOI in mm.)	Kanamycin (ZOI in mm.)
01	40	Leaf	12	26
		Stem	10	
02	80	Leaf	12	28
		Stem	13	
03	120	Leaf	20	29
		Stem	16	
04	160	Leaf	20	30
		Stem	21	
05	200	Leaf	25	32
		Stem	22	

Zone of Inhibition (ZOI)





This is an initial step to control *X.citri* under in-vitro condition. The antibacterial activity of *M. paniculata*(Methanolic extract) showed different results on different level of concentration (40µg/ml, 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml) but the maximum zone of inhibition was found on 200 µg/ml that is 25mm(leaves). On the same concentration the positive control Kanamycin gave 30mm zone of inhibition. The findings of Leksomboon *et al*., (2001) have been revealed that *X. axonopodis* pv.*citri* was inhibited against *H. subdariffa, P. guajava, S. pinnata,* and *T. indica* reduced the canker incidence varying from 18% to 52%. Antibacterial activity of vitex negundo against *X. campestris* pv. *citri* was reported by Devi *et al*., (2014) and observed that the chloroform and cow extract showed excellent zone of inhibition 14mm and 16mm respectively. Deshmukh *et al*., (2014) also worked on *X.citri* using agar well diffusion method and found the widest zone of inhibition that is 14mm. Jadhav and Deobhankar (2013) studied that *A. sativum, E. officinalis, E. globus* and *A. indica* showed highest antibacterial activity against *X.citri*. The highest zone of inhibition is 24.4mm (*E. officinalis*). The comparison of the stem of *M. paniculata* and leaf extracts, leaf extracts were effective against tested bacterial strains. The present study is an important attempt to develop plant based bactericides which are ecofriendly or beneficial to the management of *X. cirri*.

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