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ORIGINAL ARTICLE

In Vitro Antibacterial Potency of Aqueous Extracts Of Medicinal Plants against *Xanthomonas vesicatoria VMB17*

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ABSTRACT
Punica granatum is a highly valued table fruit grown in various parts of the world as it has great commercial and nutritive value and the entire tree is of great economic importance. Pomegranates are not only used for eating but also for other purposes. With the increased hectarage and adoption of improved cultural practices, a number of diseases have assumed greater importance during recent years and need proper attention for the control to avoid losses. Pomegranate crop is attacked by several bacterial pathogens. Bacterial infections involve leaf spots and oily spot disease that are caused by Xanthomonas. Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. In the present study, antibacterial potency of three medicinal plants Viz. Azadirachta indica, Pongamia pinnata and Ricinus communis have been detected on Xanthomonas vesicatoria VMB17 by Agar well diffusion assay. Among the different plants used for antibacterial activity, aqueous extracts of Azadirachta indica plant, were shown significant antibacterial activity against the pathogen Xanthomonas vesicatoria VMB17.
Keywords: *Punicagranatum, Xanthomonas vesicatoria, Plant extract, Antibacterial activity.*

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INTRODUCTION

Plant disease is one of the vital causes by reducing quantity and degrading quality of the agricultural products. Disease is impairment to the normal state of the plant which modifies or interrupts it's very important functions such as photosynthesis, pollination, transpiration, germination, fertilization, etc. The emergence of plant diseases has become more common nowadays, as factors such as climate and environmental conditions are more unsettled than ever. Plant diseases are frequently caused by bacteria, fungi, and viruses. Also there are other diseases which are caused by unfavorable environmental conditions. There are numerous characteristics and behavior of such plant diseases in which many of them are merely distinguishable. Pomegranate (Punicagranatum), so called "fruit of paradise."

The fruit is grown for it's attractive, juicy, sweet-acidic, and fully luscious grains called 'Arils'. The fruits are mainly used for dessert purposes. Insect pests and diseases are the major problems that threaten pomegranate cultivation. These necessitate careful diagnosis and timely handling to protect the crops from intense losses. In pomegranate plants, diseases can be found in several parts such as fruit, leaves and stem. Major diseases that affect pomegranate fruit are bacterial blight (Xanthomonas species), anthracnose (*Colletotrichum gloeosporoides*), and wilt complex (*Ceratocystis fimbriata*).

In recent years, there has been a major thrust on residue free organic pomegranate production. Taking the task into consideration, efficient bioagents need to be explored to fit into the management schedule. Many times, plant nutrients also play an important role in susceptibility or resistance mechanism of the host to different pathogens. Research on this aspect needs to be triggered to find out the actual role of plant nutrients in bacterial blight development/suppression.

In the present investigation, antibacterial evaluation of aqueous extracts of *Azadirachta indica*, *Pongamia*

pinnata and *Ricinus communis* plants have been performed on *Xanthomonas vesicatoria* VMB17 by Agar well diffusion assay.

MATERIALS AND METHODS

Identification of Bacterial Pathogen

Molecular identification of the bacterial pathogen

Molecular identification of the promising bacterial isolate was carried out by 18S rRNA were sequenced [1] at National Center for Cell Sciences, University of Pune Campus, Pune, Maharashtra, India.

Phylogenetic analysis:

The generated sequences were analyzed at the National Center for Biotechnology Information Bethesda, MD. www.nbi.nlm.nih.gov/BLAST for closed homology using the BLASTn algorithm. The related sequences for the isolates downloaded from the NCBI database were aligned by using CLUSTAL X2 multiple sequence alignment tool, the Phylogenetic evolutionary history was inferred using the Neighbor Joining Method analysis [2]. Phylogenetic analyses were conducted in MEGA 4.0. Phylogenetic tree building was performed using MEGA 4.0 [3].

Collection of Different Medicinal Plant Parts

Different medicinal plant *Viz. Azadirachta indica, Ricinus communis and Pongamia pinnata* were collected from various sources. Healthy, fresh, disease free plant parts *Viz*. root, stem, leaves and bark were cut with the help of sterile scissor and knife. It was kept in polythene bag and closed it securely. Afterwards, all these plant parts were brought to the laboratory and thoroughly washed with the distilled water. These parts of the plants were shade dried and then powdered with the help of waring blender. These powdered forms of plants were used for the preparation of solvent extracts.

Collection of different medicinal plant parts

Different medicinal plants *Viz*. *Azadirachta indica*, *Ricinus communis* and *Pongamia pinnata* was collected from various sources. Healthy, fresh, disease free plant parts *Viz*. root, stem, leaves and bark were cut with the help of sterile scissor and knife. It was kept in polythene bag and closed it securely. Afterwards, all these plant parts were brought to the laboratory and thoroughly washed with the distilled water. These parts of the plants were shade dried and then powdered with the help of waring blender. These powdered forms of plants were used for the preparation of solvent extracts.

Preparation of Medicinal Plant Extracts

Preparation of Aqueous Extract of Selected Medicinal Plant Parts

Twenty gram of thoroughly washed fresh plant parts *Viz*. root, stem, leaves and bark were macerated with 100ml sterile distilled water taken separately in a waring blender for 10 minutes. The crush of each plant was then filtered through muslin cloth having double layer and then centrifuged (Remi Centrifuge model R-8C DX+R-81A) at4000 rpm for 30 minutes. The supernatant was then filtered just before subjecting it to antibacterial activity assay [4].

Preparation of Solvent Extracts of Selected Medicinal Plants

Twenty grams of dried powder of each plant was filled in the thimble and extracted consecutively with the solvents *Viz*. methanol, ethanol and chloroform by using a Soxhlet extractor (Soxhlet Complete Borosil, Code 3840) for 48 hours. All these extracts were separately prepared in Soxhlet extractor. Further, all these extracts were concentrated using a rotary flash evaporator (Superfit Rotary Vacuum Flash Unit PBU-6D) and preserved at 4^0C in airtight bottles for further use. Afterwards, all these extracts were subjected to antibacterial activity assay [4].

Determination of Antibacterial Activity

The antibacterial activity of aqueous extracts, solvent extracts and the mixture of all plant extracts under study were determined by agar well diffusion method [5] on the sterile nutrient glucose agar medium. Cell density was adjusted to 10^6 -10^7 CFU/ml on the basis when culture reaches 0.1 optical units at 600nm with a spectrophotometer [6]. The inoculum containing 10^6-10^7 CFU / ml of 72 hours old culture of *Xanthomonasvesicatoria VMB17* was spread inoculated on medium with the help of sterile glass spreader. Wells were prepared in sterile nutrient glucose agar plate by using sterile cork borer (5mm). 100µl of aqueous extract and solvent extract was put in the wells prepared in the inoculated plates.

Four plant extracts which showed noteworthy antibacterial activity against all the pathogens were selected to prepare its mixture and used to study its antibacterial potential against pathogens. Similarly 100µl of mixture of all selected plant extracts was then put in the well made in the inoculated plate. 100µl of solvent was placed in a blank well made in sterile nutrient glucose agar plate. It was done to determine the antibacterial activity of solvent against the pathogen. All the plates were kept in the refrigerator at 4^0C for 20 minutes for diffusion and incubated for 72 hours at 30^0C. The diameter of the zone of growth inhibition around the wells was measured in millimeter (mm) [4]. All the experiments were performed in triplicates.

RESULTS

Three medicinal plants *Viz. Azadirachtaindica, Ricinus communis* and *Pongamiapinnata* were tested against

Xanthomonas vesicatoria VMB17 by agar well diffusion assay. *In Vitro* evaluation of different medicinal plants extract *Viz*. aqueous, ethanolic and methanolic was taken to test the antibacterial evaluation.

Phylogenic Analysis of VMB17:

The phylogenetic tree was constructed by using the Neighbour joining method by Kimura – 2 parameters with 1000 replicates in MEGA 4.0. According to the sequencing similarities and multiple alignments, the present isolate was identified. The sequence obtained of the present isolate has been deposited in DNA Databank of Japan (DDBJ) and accession number obtained (**Figure 1)**.

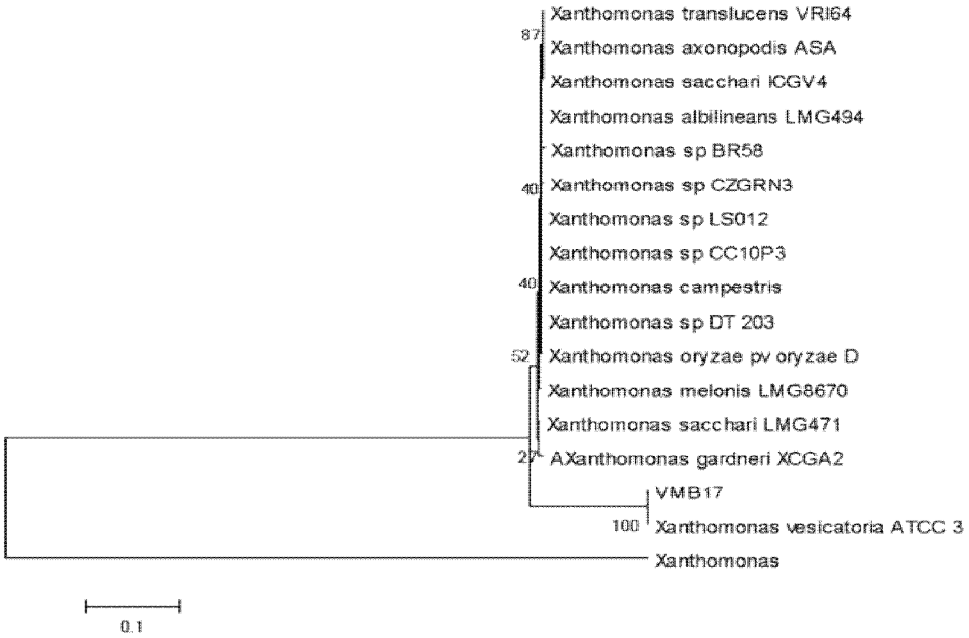


Figure 1: Phylogenetic placement of VMB17 (DDBJ Accession Number LC530855) based on 16S rRNA analysis

Phylogenic tree of *Xanthomonas vesicatoria VMB17*. Phylogenetic analysis of 16s rRNA gene sequence of *Xanthomonas vesicatoria VMB17*. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1,000 replicates. The scale bar (0.1) indicates the genetic distance.

In Vitro* evaluation of Antibacterial Potential of Some Medicinal Plants against *Xanthomonas vesicatoria VMB17

All the aqueous plant extract of three medicinal plants *Viz*. *Azadirachta indica, Ricinus communis* and *Pongamia pinnata* showed antibacterial activities against the test bacterial pathogen *Xanthomonasvesicatoria VMB17* with some showing better antibacterial activities than others. Whereas the control (only distilled water means without medical plant extract) of all the extracts were also taken for anti bacterial test evaluation against *Xanthomonas vesicatoria VMB17*.

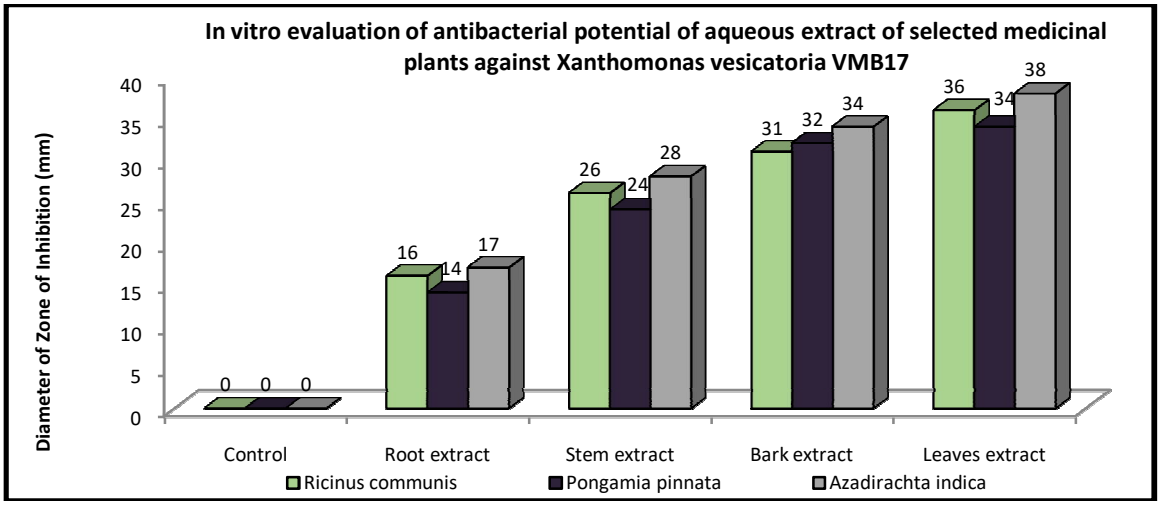
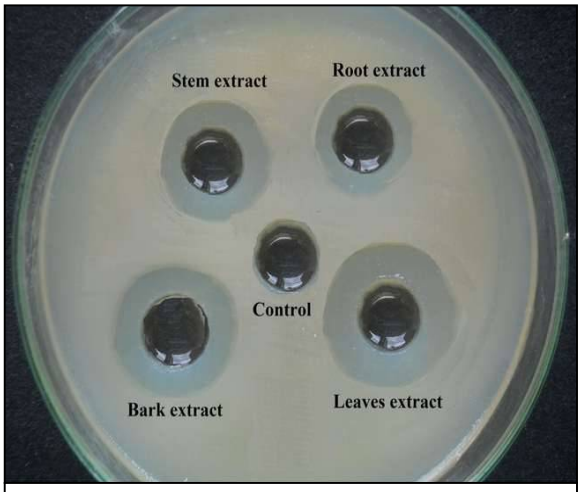
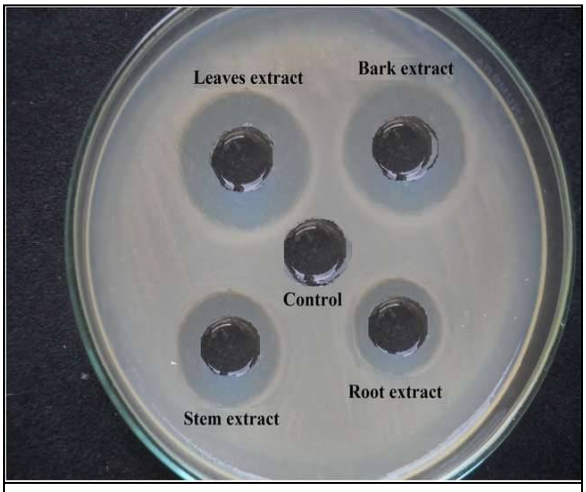


Figure 2: *In Vitro* Evaluation of Antibacterial Potential of Aqueous extract of selected medicinal plants against *Xanthomonas vesicatoria VMB17*

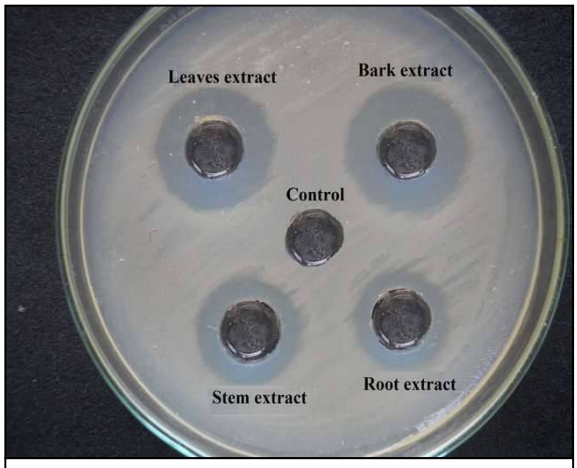
It is found from the **Figure 2** that the promising fungal isolate *Xanthomonas vesicatoria VMB17* shows the maximum zone of inhibition i.e. 38mm to aqueous leaves extract of *Azadirachta indica* followed by bark extract, stem extract and root extract which is 34mm, 28mm and 17mm respectively. Similarly, the promising fungal isolate VMB17 shows maximum zone of inhibition i.e. 34mm to aqueous leaves extract of *Pongamiapinnata* followed by bark extract, root extract and stem extract which is 32mm, 24mm and 14mm respectively. The promising fungal isolate VMB17 shows maximum zone of inhibition i.e. 36mm to aqueous leaves extract of *Ricinuscommunis* followed by bark extract, root extract and stem extract which is 31mm, 16mm and 26mm respectively.Among the different plants used for antibacterial activity, aqueous extracts of *Azadirachta indica* plant, were shown significant antibacterial activity against the pathogen *Xanthomonas vesicatoria VMB17*.



Aqueous extract of *Azadirachta indica* against *Xanthomonas vesicatoria VMB17*



Aqueous extract of *Pongamia pinnata* against *Xanthomonas vesicatoria VMB17*



Aqueous extract of *Ricinus communis* against *Xanthomonas vesicatoria VMB17*

Photoplate 1: *In Vitro* evaluation of antibacterial potential of aqueous extract of selected medicinal plants against *Xanthomonas vesicatoria VMB17.*

DISCUSSION

Pomegranate crop is prone to the number of diseases amongst which bacterial leaf blight caused by *Xanthomonas species* is a serious problem and crisis to pomegranate production due to its high epidemic potential. The disease influences all the above ground parts of the plant including flower, leaves, stem, twigs, buds and fruits, but it is more destructive when the fruits are infected. Plant diseases and their management is an important concept in order to increase agricultural production. Numbers of pesticides are generally used in plant disease management in many developed and developing countries. Pomegranate, the boon commercial fruit crop to the farmer, turned as a big bane after the outbreak of bacterial blight. In recent years, bacterial blight of pomegranate became a serious disease, which is

caused by *Xanthomonas species.*

The methanol extracts of *Chromolaena odorata* having antimicrobial activity against *Escherichia coli, Xanthomonas vesicatoria, Ralstoniasolana cearum* and *Staphylococcus aureus* by using agar disc method in which diameter of inhibition zone of 10mm, 9mm, 12mm and 12mm respectively. The Minimum inhibitory concentration (MIC) value of these extract for these clinical bacteria ranged between 0.35mg/ml to 4.0mg/ml and 0.25mg/ml to 4.0mg/ml for phytopathogenic bacteria [7].

The antagonistic effect of *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Lactobacillus spp.* on the growth of *Xanthomonas axonopodispv. Punicae*by both inhibition assay and dual culture plate method and observed that none of these antagonists were inhibitory to the growth of the *Xanthomonas axonopodispv. Punicae* [8].

The antibacterial activity of aqueous, solvent extracts and isolated constituents of *Acacia nilotica* leaves against three phytopathogenic *Xanthomonas* pathovars *Viz*., *Xanthomonas axonopodis* pv. *Phaseoli Xanthomonas campestris*pv. *Vesicatoria* and *Xanthomonas axonopodis*pv. *malvacearum,* associated with angular leaf spot of cotton, common blight of bean and bacterial spot of tomato. Aqueous, methanol and ethanol leaf extracts of *Acacia nilotica* showed significant antibacterial activity against all the pathovars of *Xanthomonas* [4].

CONCLUSIONS

The upshot of present investigations would assist farmers to use an effective method by using native medicinal plants for the control of the bacterial blight disease of pomegranate in cost competitive and eco-friendly style.

CONFLICT OF INTREST

There is no conflict of interest among the authors.

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