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Anti-microbial activity of some Botanicals against the *Xanthomonas axonopodis* p.v. *punicae* in Pomegranate

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Abstract
Pomegranate (*Punica granatum* L.) is the important crop cultivated throughout the world well known for its refreshing fruits, nutritional, and medicinal properties. The crop yield of pomegranate is greatly affected by bacterial blight causing *Xanthomonas axonopodis* p.v. *punicae.* Treating bacterial blight with chemicals and antibiotics is not very effective because of the high cost expenses and less result in eliminating the infection completely and safely, leaving farmers with heavy economic losses. In the present study 23 plants extracts with 3 solvents *viz.*, Hexane, Chloroform, and Methanol were evaluated for their antibacterial activity against the pathogen *Xanthomonas axonopodis* by Well Diffusion method and Minimum Inhibition Concentration (MIC) methods. In view of discovering new plant products that effectively inhibit the growth of the pathogen in a safe and economical way. Among 23 plants, plant extracts of *Albezzia lebbeck*, *Catharanthus rosea* and *Calotropis gigantea* showed antibacterial activity. In these three *Albezzia lebbeck* has given the highest activity at lowest concentration *i.e.*, 10 mg ml^{-1} with 15 mm diameter of inhibition zone against the tested pathogen *Xanthomonas axonopodis* p.v. *punicae.*

Keywords: pomegranate, bacterial blight, *Xanthomonas axonopodis* p.v. *punicae*, plant extracts, anti-bacterial activity

1. Introduction
Pomegranate (*Punica granatum*) belongs to the family *Punicaceae* is an ancient fruit crop of India and also regarded as the fruit of paradise. It is native to Iran but extensively cultivated in Mediterranean regions especially in Spain, Morocco, Egypt, Japan, USA, USSR, Bulgaria, and Southern Italy. It is regarded as the "vital cash crop" of an Indian farmer and is grown in an area of 1.3 hectare with a production of 11.0 lakh tones (Jadhav and Sharma, 2009) [5].

The fruits of Pomegranate are known to possess pharmaceutical and therapeutic properties. The fruit has a wide consumer market and high economical value for its attractive, juicy, sweet, acidic and refreshing arils, nutritional and medicinal properties and is a good source of carbohydrates and minerals such as Calcium, Iron and Sulphur. It is rich in Vitamin-C with Citric acid as the predominant organic acid (Malhotra *et al.*, 1983) [8]. The loss in the production of yield is very high annually due to microbial infections and pests along with several physical and chemical constraints. Among the diseases infecting pomegranate, the bacterial blight disease popularly known as "bacterial blight, Nodal blight and oily spot" caused by *Xanthomonas axonopodis* p.v. *punicae* (Hingorani and Singh, 1959) [4] is a major biological production constraint (Chand and Kishun, 1991) [2] noticed that the epidemic of bacterial blight of Pomegranate is causing 60 to 80% losses at Indian Institute of Horticultural Research (IIHR) experimental plots. Now it is learnt that, bacterial blight of Pomegranate is a wide spread and major production constraint. The disease prevailed in all the seasons with varying degree of severity. Studies on cultural, morphological, physiological and biochemical features of the pathogen are of immense use in understanding the nature of pathogen. Not much work has been done on these aspects but felt necessary to do so.

The pathogen can infect at any stage of growth in the life of plant. The damage is observed on fruits which develop black oily spots later become completely black, then splits into and dries off. In advanced stage of infection, tissue necrosis occurs on leaves and twigs. In the case of fruits, the disease develops into 'V' or 'Y' shaped cracks (Yenjeerappa, 2009) [15] and later the fruit becomes completely black and dries off. Spraying various chemical formulation of antibiotics like Streptomycin, Copper Oxy Chloride, Bordeaux mixture, Bromopol to control the disease produces limited success (Mondal and Mani, 2012) [10]. Regular use of chemicals in agricultural land causes killing of natural and useful flora and fauna of the soil, increase in development of resistance in plant pathogen against the chemicals and residual toxicity remains in plant and animals. To overcome these problems there is a worldwide growing

interest in the development and utilization of harmless, economical, and ecofriendly material for pathogen control (Madhiazhagan *et al*., 2002) [7].

In order to reduce these of the harmful chemical pesticides, plant extracts are widely screened for its antimicrobial activity against several pathogenic microorganisms with the aim of drug development (Britto *et al*., 2011) [1]. Plant disease management measures are eco-friendly, effective and economical in this manner in comparison to chemical or physical methods.

2. Material and Methods

2.1 Isolation and identification of pathogen

Fruits samples infected with bacterial blight were collected from orchard of Solapur, and identified by the National Research Centre on Pomegranate, Solapur, Maharashtra, India. The isolated bacteria in fruit lesions were confirmed by performing Ooze test. Ooze test is performed by cutting 1cm of the infected portion of the fruit and the outer surface was removed and sterilized with 0.1% Mercuric Chloride solution for 1 minute and later washed several times with sterile distilled water. Then the infected portion was squeezed gently with a sterile scalpel into a sterile saline by placing in a petri dish. Later the suspension was inoculated into a sterile petri dish with Yeast Extract Calcium Carbonate Glucose Agar medium (Yenjeerappa, 2009) [15] at pH at 6.5 and were incubated at 30°C for 48 hrs. After the incubation typical mucoid yellow color colonies were selected and screened for morphological and bio-chemical characteristics according to Bergy's manual of determinative Bacteriology and identified as *Xanthomonas axonopodis* p.v. *punicae*.

2.2 Preparation of Plant extracts

The twenty-three plants selected for this study were mostly weeds. These plants were chosen in order to reduce the cost of the plant material during industrial production of the pesticides in case significant activity is found in them.

2.3 Collection of plant material

Twenty-three healthy plants (Table-1) were collected based on the information from literature and through field observation. The plant materials were collected from Visakhapatnam District, Andhra Pradesh, India. Taxonomic identification of the plants was done by the reference to the flora of Visakhapatnam by Venkateshwarlu *et.al*. (1972) [14] as well as comparison with the authenticated herbarium specimens available in the Department of Botany, Andhra University. The collected material was washed thoroughly under running tap water and finally with sterile distilled water. Then the materials are air dried on sterile blotter under shade (Khokar *et al*., 2012) [6].

2.4 Solvent Extraction of Plant Material

The completely shade dried plant material (the plant parts used in this study were selected on the basis of its importance in traditional medicine and review of literature) was coarsely powdered using electric blender. Then it was allowed to soxhilate extraction separately and successively with Hexane, Chloroform and Methanol solvents. Later the soxhilate material was subjected to distillation to remove the solvent. After distillation different extracts obtained were concentrated with Rotary evaporator and brought to complete dryness over a water bath to yield the crude extract and preserved at 4°C for further use (Raghavendra *et al*., 2006) [11].

2.5 Anti-Microbial activity of the plant extracts

The anti-bacterial activity of plant extracts was determined by Agar Well Diffusion method (Schaad *et.al*., 1992) [13] to screen for antibacterial activity of the Hexane, Chloroform, and Methanol solvents of 23 plant extracts. Dimethyl sulfoxide (DMSO) is used as solvent to dissolve the crude extract of plant.

2.6 Preparation of media and plates for Agar diffusion method

In order to determine the antibacterial activity of plant extracts Agar Well Diffusion method was performed using Yeast Extract Calcium Carbonate Glucose Agar as nutrient media. 100 ml of Yeast Extract Agar medium was taken and autoclaved at 121°C at 15lbs pressure. The media was removed and brought to 40°C. 1ml of the tested bacterial suspension of *Xanthomonas axonopodis* p.v. *punicae* (equivalency to 0.1ml of 0.5 McFarland standards) [9] was added to the Yeast extract agar nutrient medium and mixed well then it is poured into petriplates and allowed to cool under strict aseptic conditions. After the solidification of media, wells were made in each petriplate with the help of cork borer of 4mm diameter. The wells of each plate were well marked with permanent marker. To determine the potential of plant extracts different concentrations ranging 500, 250, 100, 50, 25 and 10 mg ml^{-1}were diluted in DMSO solution and 40 µl of plant extract was poured into each well according to the markings on the petriplates.

2.7 Minimum Inhibitory Concentration (MIC)

In order to determine Minimal Inhibitory Concentration (MIC) bacterial isolates were diluted in 1ml Yeast extract broth to get 0.5 McFarland 0.1 ml turbidity. 1ml of various concentrations of *Albezzia lebbeck* extract ranging 10, 9, 8, 7, 6 and 5 mg ml^{-1}were added into respective test tubes marked. Containing 0.9 ml of Yeast Extract Broth each according to the concentrations into each tube 50 µl of bacterial suspension was added. One tube of 10 ml Yeast Extract Broth with no plant extract is taken as test control, and one tube with 10 ml of Yeast Extract broth was taken as blank. One tube with 9 ml of broth is taken and 1ml of Streptomycin (5 mg ml^{-1} Concentration) was added as standard. Then the tubes were incubated at 30°C for 24hrs. After incubation MIC was evaluated by observing the tubes for the lowest concentration of the plant extract that is inhibiting the visible growth of bacteria. The turbidity indicating the growth of the pathogen was measured by using Spectrophotometer. The MIC value of *Albezzia lebbeck* was obtained at 0.8 mg ml^{-1} concentration.

3. Results

After incubation of the plates for 24 hours at 30°C results were observed and noted. The results of the present study show that the methanolic extract of *Albezzia lebbeck* has exhibited highest activity with 15mm diameter of inhibition zone at the lowest concentration of 10 mg ml^{-1}, whereas Chloroformic and Hexane extracts does not show any antibacterial activity. Hexane extract of *Catharanthus roseus* showed moderate activity at 100 mg ml^{-1} concentration with inhibition zone of 13mm diameter. The Hexane and Methanol extracts of *Calotropis gigancea* has showed lowest activity at 100 mg ml^{-1} concentration with inhibition zone 10mm diameter. Antibiotic Streptomycin at 5 mg ml^{-1} is used as standard showed activity with the inhibition zone of 16mm diameter. The mean results of antibacterial activity of different solvents of *Albbezia lebbeck*, *Catharanthus roseus*

and Calotropis gigantean along with standard antibiotic Streptomycin are summarized in Table -2 with standard errors.

4. Discussion

Methanol extract of *Alebezzia lebbeck* exhibited good antifungal activity against *Fusarium oxysporum, f.spcapsici* showed the lowest MIC (mg ml^{-1}) Dewa *et al*., (2012) [3]. Chloroform and petroleum ether fractions showed potent activity against *Asperigillus niger, penicillium sp* and *T. harzianum* (Roushanul *et al*., 2009) [12]. However, not much work was done regarding Bacterial Blight of Pomegranate, especially on bacterial pathogen *Xanthomonas axonopodis* p.v. *punicae.*

5. Conclusion

Bacterial blight of Pomegranate is a major disease of the Pomegranate crop that affects around 50% of the production. The current scenario forces to find an ecofriendly and effective method to control the disease. The present study highlights the activities of Chloroform, Methanol and Hexane extracts of *Albezzia lebbuck, Catharanthus roseus, and Calotropis gigantea* against the pathogen *Xanthomonas axonopodis* p.v. *punicae*among which the crude Chloroform extract of *Albezzia lebbuck* showed significant activity inhibiting *Xanthomonas axonopodis* at lower concentrations, when compared with the standard drug Streptomycin. These results can further extrapolated to find an economical ecofriendly and effective method to treat bacterial blight of Pomegranate.

Table 1: List of the plants used in this present study

S. No.	Botanical name	Family	Local/ vernacularname	Plant parts used
1.	*Abutilon indicum (Link)Sweet*	*Malvaceae*	Duvvenabenda	Whole plant
2.	*Accacia nilotica (L.) Wild.ex.Delile*	*Fabceae*	Mullatumma	Bark
3.	*Alangium salviifolium (L. f.) Wangerin*	*Cornaceae*	Urgu	Bark
4.	*Acalypha indica L.*	*Euphorbiaceae*	Haritamanjari, Kupichettu	Whole plant
5.	*Albbezia lebbuck (L.) Benth.*	*Fabaceae*	Girishamu	Leaves
6.	*Phyllanthus emblica L.*	*Rutaceae*	Vusiri	Leaves
7.	*Boerhaavia diffusa L.*	*Nyctaginaceae*	Erragalijeru, atikamanidi	Whole plant
8.	*Calotropis gigantea (L.) W.T.Aiton*	*Asclepiadaceae*	Tellajilledu	Whole plant
9.	*Chromoleana odorata (L.)King & H.E..Roins*	*Astaraceae*	Christmasbush	Whole plant
10.	*Catharanthus roseus (L.) G.Don*	*Apocynaceae*	Billaganneru	Whole plant
11.	*Cassia alata (L.) Roxb*	*Fabaceae*	Sennaalata	Leaves
12.	*Datura metel L.*	*Solanaceae*	Ummetha	Whole plant
13.	*Elytraria acaulis (L.fil.)*	*Acanthaceae*	Yeddadugu	Whole plant
14.	*Glycosmis pentaphylla (Retz) DC.*	*Ruteaceae*	Orange berry	Leaves
15.	*Hugonia mystax L.*	*Linaceae*	Venopa, climbing flax	Bark
16.	*Hybanthus enneaspermus (L.)*	*Violaceae*	Ratnapurusha	Whole plant
17.	*Malvastrum coromandellianum (L.) Garcke,*	*Malvaceae*	Broom weed	Leaves
18.	*Melia azadirachta L.*	*Tagetuserecta*	Thurakuvepa	Leaves
19.	*Polyalthia longifolia Sonn.*	*Annonaceae*	Asoka chettu	Asoka chettu
20.	*Ruellia tuberose L.*	*Acanthaceae*	Chitapatakayalamokka,	Whole plant
21.	*Solanum sisymbrifolium Lam.*	*Solanaceae*	Sticky night shade	Whole plant
22.	*Senna occidentalis (L.) Link,1829*	*Fabaceae*	Kasinda	Whole plant
23.	*Tinospora cordifolia (Thunb) Miers*	*Minispermaceae*	Tippateega	Leaves

Table 2: Antibacterial activity of plant extracts on growth of *Xanthomonas axonopodis* p.v. *punicae*

Plant list	500mg/ml			250mg/ml			100mg/ml			50mg/ml			25mg/ml			10mg/ml			
	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	
Alezzia lebbuck	Nil	Nil	24±2	Nil	NIl	23±2	Nil	Nil	22±1	Nil	Nil	18±1	Nil	Nil	16±1	Nil	Nil	15±2	Highest
Catharanthus roseus	15±1	Nil	Nil	14±1	Nil	Nil	13±2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Moderate
Calotropis gigantea	10±2	Nil	12±1	9±1	Nil	11±1	8±1	Nil	10±2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	low

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