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Research Article

Screening of antibacterial activity of *Ricinus communis* L. leaves extracts against *Xanthomonas axonopodis* pv. *punicae*

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Abstract

In present study efficacy of *Ricinus communis* L. for growth inhibition of *Xanthomonas axonopodis* pv. *punicae*, causative agent of Bacterial blight of Pomegranate (*Punica granatum* L.) was determined. The aqueous extract and solvent extracts of leaves of *Ricinus communis* L. were tested for its antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* by agar well diffusion method. The methanol and ethanol extracts showed significant antibacterial activity against the pathogen. Minimum Inhibitory Concentration was determined by broth dilution assay. Further investigation was carried out to determine the possible bioactive components present in leaves of *Ricinus communis* using qualitative phytochemical analysis.The results of present study showed that *Ricinus communis* has potential to control the growth of *Xanthomonas axonopodis* pv. *punicae*.

Keywords: *Ricinus communis*, antibacterial activity, *Xanthomonas axonopodis* pv. *punicae*, Minimum Inhibitory Concentration, Phytochemical analysis.

Introduction

The Pomegranate (*Punica granatum*) is an ancient, medicinal fruit and cash crop of Maharashtra. It is also grown commercially in other states of India viz. Karnataka, Andhra Pradesh, Rajasthan, Gujarat and Tamil Nadu (Manjula *et al*.,2009).Pomegranate has high nutritional, medicinal value and health benefits (Julie Jurenka,2008;Aallwyn *et al*.,2014). Total production of pomegranate fruit in India is about 8 lakhs tonnes per annum (Pawar *et al*.,2013).Since past few years, production and yield quality of Pomegranate is affected by bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*. Due to bacterial blight disease farmers across the state have lost at least 60% of their crop, as infection rate of upto 100% has been reported in some orchards. The disease has become increasingly serious threat for pomegranate growers of Andhra Pradesh, Maharashtra and Karnataka states of Indian subcontinent (Kumar *et al.*, 2009).The symptoms of bacterial blight were observed on the leaves stems and fruits as small (2 to5 mm) in size, irregular, prominent water soaked spots, which later become black often leads to the breaking of branches and cracking of fruits , reducing the market value of the fruit (Rangaswami,1962; Manjula and Khan, 2002; Sheikh,2006; Bengi and Ravikumar,2009).

Presently bacterial blight of pomegranate disease management includes spraying chemicals and antibiotics viz., *Streptocycline*, Copper Oxychloride, Ampiclox (Lokesh *et al*.,2014).Bordeaux mixture, Captan ,Copper oxychloride, Copper hydroxide , Bromopol, Streptocycline (Raghuwanshi *et al*.,2013) was recommended.

Use of chemicals in agricultural land causes detrimental effect on natural flora and fauna of soil (Madhiazhagan *et al*.,2002;Shanthi *et al*., 2011).To overcome this problem search for alternative method is need of hour. Medicinal plants which are potential source of various phytochemicals having antimicrobial, antioxidant, anticarcinogenic activity (Kapoor and Mishra, 2013).

Ricinus communis belongs to family Euphorbiaceae**,** which is commonly known as castor. It is a small soft wooden tree which is found all over the India and also in tropics and temperate regions of the world. Ancient Indian literature considers all plant parts *viz*. bark, leaves, flowers, seed, oil etc. to be used as potential source of medicinal substances (Rana *et al.*,2012 Hogade *et al*.,2013).The leaf of plant is reported to possess antioxidant, antifungal and antibacterial activity (Naz and Bano,2012). All these uses are due to the presence of certain phytoconstituents in the plant (Jena, 2012).Hence present study is focussed on evaluation of antibacterial potential of herbal extracts of *Ricinus communis* leaves against *Xanthomonas axonopodis* pv. *punicae*.

Materials and Methods

Isolation and Identification of the pathogen from lesions of diseased fruit

Diseased Pomegranate fruits showing typical symptoms of bacterial blight were collected from field located in Solapur District. The pathogen was isolated by dilution plate technique (Yenjerappa,2009).The presence of bacteria in fruit lesions was confirmed by performing ooze test (Singh *et al.*,2015). The suspension was serially diluted and grown on Nutrient Glucose Agar medium. The inoculated plates were incubated at 30ºC for 72 hours. The isolated pathogen was subjected to study its morphological and biochemical characteristics (Bora and Kataki, 2014). For pathogenecity test, Koch's postulates were followed to prove pathogenic nature of *Xanthomonas* isolate (Shaad, 1992) and molecular identification of pathogen was done on the basis of 16S rRNA sequencing.

Collection and Authentication of plant material

Fresh healthy, matured, disease free leaves of *Ricinus communis* were collected from Kamber lake area of Solapur city, Maharashtra. The collected plant parts were identified from Department of Botany, Walchand College of Arts and Science, Solapur, Maharashtra.

Preparation of aqueous extract

25g leaves of *Ricinus communis* were thoroughly washed with running tap water, shade dried, chopped and were macerated separately in pestle and mortar with 100 ml of distilled water and kept for 24 hours at room temperature. The mixture was filtered and centrifuged at 4000 rpm for 5 minutes. The supernatant was evaporated and stored at 4ºC (Nidaullah *et al.*,2010).

Preparation of solvent extracts

Twenty-five grams of the leaves powder of *Ricinus communis* L. extracted successively with petroleum ether, benzene, chloroform, ethanol and methanol using a Soxhlet extractor. All the extracts were concentrated using rotary flash evaporator (Superfit Rotavap Model PBU-6D) and preserved at 4ºC in airtight bottle until further use (Raghavendra *et al.*,2006).

Antibacterial activity assay

Antibacterial activity of aqueous extract, solvent extracts was determined by Agar well diffusion method (Cruickshank *et al.*,1975) on sterile Nutrient Glucose (NG) agar medium. Similarly NG medium plate carried a blank well with solvent served as a positive control. The bactericidal effect of 100 µl of 0.1% aqueous, methanol, ethanol, chloroform, petroleum ether extracts of *Ricinus communis* L. leaves assayed against phytopathogenic *Xanthomonas axonopodis* pv. *punicae*. The antibacterial activity of aqueous and solvent extracts against isolate was carried out by agar well diffusion method on Nutrient Glucose Agar medium.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of solvent extracts against isolated pathogen was determined by broth dilution method. For broth dilution tests, 0.1ml of standardized suspension of isolated bacterial culture (10^6CFUs/ml) was added to each tube containing different concentrations of the extracts (100-1000 µl/ml) and incubated for 72 hours at 30ºC.The tubes were incubated at 30^0c for 72 hours and checked for turbidity. Minimum inhibitory concentration was determined as the highest dilution of the extract that showed no visible growth.

Phytochemical analysis

The methanol and ethanol leaves extracts of *Ricinus communis* L. showing antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* were subjected to qualitative phytochemical analysis by following the procedure of Raaman, 2006.

Results

Isolation and identification of pathogen

After 72 hours incubation at 35ºC on Nutrient Glucose agar, typical mucoid ,smooth ,opaque yellow coloured colony showing characters similar to *Xanthomonas* spp. was isolated and identified as *Xanthomonas* spp. Microscopic observations showed that isolated bacterium appear in singly and in pairs, Gram negative, capsulated, motile rods. The results of biochemical tests showed positive for acid production from glucose, fructose, mannose and citrate. It is positive for Hydrogen sulphite production, Starch hydrolysis, Catalase and Oxidase production while negative for Urease production, Gelatine liquefaction (Gargade and Kadam, 2015).

The results of pathogenecity test showed development of oily black spots on the leaf surface after 10 days incubation. The organism re-isolated from artificially inoculated plant yielded an organism similar to one used in the inoculation experiment. For molecular identification of isolate based on 16S rRNA sequencing was carried out in Rajiv Gandhi Centre for Biotechnology, Kerela, India. The sequence of 16S r RNA of this isolate has been deposited into GeneBank under accession number (**KP168824).**

Antibacterial activity

Among various extracts, methanol and ethanol extract of *Ricinus communis* leaves exhibited 24±2.08 mm and 18±1.52 mm diameter of zone of growth inhibition against *Xanthomonas axonopodis* pv. *punicae* respectively (Table.1).Aqueous, ether, benzene and chloroform extracts showed no significant antibacterial activity against *Xanthomonas axonopodis* pv. *punicae*.

Minimum Inhibitory Concentration

The MIC of methanol extract required for inhibition of growth of *Xanthomonas axonopodis* pv. *punicae* is 500 µg/ ml while for ethanol extract is 1000 µg/ ml.

Phytochemical Screening

The results of phytochemical screening revealed that the methanol and ethanol extract of *Ricinus communis* leaves contain terpenoid, phenolic compounds, saponins, steroids, volatile oils, carbohydrates and glycosides were absent. (Table.2)

Discussion

Bacterial Blight on Pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*, is controlled by spraying various formulations of antibiotics and chemicals viz., *Streptocycline*, Copper Oxychloride, Ampiclox, Bordeaux mixture, Captan ,Copper hydroxide , Bromopol (Raghuwanshi *et al*.,2013; Lokesh *et al*.,2014).

Medicinal plants known to posses various secondary metabolites having tremendous antimicrobial potential (Satish *et al*.,1999; Raghavendra *et al*.,2006; Raveesha *et al*.,2007; Jadhav and Deobhankar, 2013;Yumlembam and Borkar,2014).The leaf of *Ricinus communis* plant is reported to possess antioxidant, antifungal and antibacterial activity (Naz and Bano,2012).The results of present study showed leaves of *Ricinus communis* having antibacterial potential can be used to control growth of *Xanthomonas axonopodis* pv. *punicae*. Hence further study is needed for screening of antibacterial activity and presence of phytoconstituents in various parts of *Ricinus comminus*. Furthermore, in vivo antibacterial activity of *Ricinus communis* leaves extracts against bacterial blight pathogen on Pomegranate needs to be determined in plants grown in fields.

Table 1: Antibacterial activity of aqueous and solvent extracts of *Ricinus communis* expressed as diameter of zone of inhibition in millimeter (mm)

Sr.No.	Plant part used	Aqueous	Ethanol	Methanol	Petroleum Ether	Benzene	chlorofom
1	Leaves	--	18±1.52	24±2.08	--	--	--
2	Solvent	--	8±0.763	6±1	2±1	--	--

Values are the means of three replicates ± standard deviation
-- absent

Table 2: Phytochemical analysis of *Ricinus communis* extracts:

Photochemical	Test used	Methanol extract	Ethanol extract
Alkaloids	Mayer's Test	++	++
Carbohydrates and glycosides	Fehling's Test	--	--
Terpenoids	Noller test	++	++
Phenolic compounds And Flavonoides	Ferric Chloride Test	++	++
Saponins	Foam test	++	++
Steroids	Libermann-Burcard Test	++	++
Volatile oils	Steam Distillation	++	++

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