

Original Research Article

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Potentiality of Extracts of Medicinal Plants against Important Plant Pathogenic Bacteria

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ABSTRACT

Bacterial diseases of plants are highly catastrophic and management is difficult. Using of chemicals are hazardous. Hence, an alternate method of control needs to be developed which is effective, cheap and eco-friendly. The water extract from the two botanicals viz., *Ocimum gratissimum* and *Tylophora asthmatica* were effective in inhibiting the growth of *Ralstonia solanacearum*, *Xanthomonas axonopodis* pv. *vignicola* and *X. campestris* pv. *Campestris* upto 1:1 dilution. At 1:10 dilution water extract of *O. gratissimum* produced inhibition zones of 22.66, 21.66 and 22.33mm against *R. solanacearum*, *X. axonopodis* pv. *Vignicola* and *X. campestris* pv. *campestris* respectively. Of the three bacteria tested, *R. solanacearum* was found to be more sensitive to *O. gratissimum* followed by *X. campestris* pv. *campestris* whereas, in cases of *T. asthmatica*, *X. campestris* pv. *campestris* was more sensitive followed by *X. axonopodis* pv. *vignicola* and *R. solanacearum*. Alcohol extract of *O. gratissimum* was effective against all the three bacteria tested upto 1:100 dilution, whereas, *O. sanctum* and *T. asthmatica* were effective upto 1:10 dilution. *Ruta graveolens* was effective upto 1:1 dilution against the three bacteria. In majority of cases *X. campestris* pv. *campestris* was most sensitive to the alcohol extracts followed by *X. axonopodis* pv. *vignicola* and *R. solanacearum* indicating that with the dilution of extract the inhibitory activity was lost.

Keywords

Medicinal plants:
Ralstonia solanacearum:
Xanthomonas axonopodis pv.
vignicola: Inhibition zone

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Introduction

Among the diseases of agricultural and horticultural crops, diseases caused by bacteria are usually very difficult to control. Frequently a combination of different methods of control is required to combat a given bacterial disease. Also, use of chemicals to control bacterial diseases has not been much successful as compared to the control of fungal diseases. The use of copper based bactericides seldom gave satisfactory control. Antibiotics used in the control have no doubt been successful in controlling the disease, however, use of antibiotics have certain drawbacks such as its prohibition cost, quick break down in nature, residual toxicity because of its systemic nature and more importantly quick

development of resistance to the antibiotics by the bacterial pathogens. Botanicals because of their natural origin are biodegradable and they do not leave toxic residues or by-products to accumulate in the environment. Several plants have thousands years of history and the non-toxicity at least at oral level is proved. Further, the research and development cost of botanical pesticides from discovery to marketing is much less compared to chemical pesticides. Therefore under this scenario, botanical pesticides seem to be ideal candidate to be exploited in management of bacterial diseases in view of the safety, renewable nature, cost effective and high target specificity apart from the fact that some plants have more

than one active principle responsible for their biological property. Hence an investigation was conducted to study the effect of extracts from few important medicinal plants against few phyto-bacteria.

Materials and Methods

Selection of medicinal plants

Medicinal plants mentioned in table 1 which were reported to contain some antibacterial constituents and being used in Indian system of medicine (Kamala Ramachandran *et al.*, 1986) were selected to screen for their antibacterial properties against three important plant pathogenic bacteria causing serious diseases of vegetable crops. These plants were collected from College of Agriculture, Mandya.

Isolation of pathogens

The three important plant pathogenic bacteria viz., *Ralstonia solanacearum*, causal agent of tomato wilt, *Xanthomonas axonopodis* pv. *vignicola* causing bacterial blight of cowpea and *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage were isolated from infected plants.

Tomato plants showing typical symptoms of vascular discoloration caused by *R. solanacearum* were collected. The presence of the pathogen in the host was confirmed by ooze test. The bacterium was isolated on solidified triphenyl tetrazolium chloride (TZC) agar medium (Kelman, 1954). The tissue from the lower part of the infected stem were cut into small pieces aseptically, and surface sterilized in 70 percent alcohol and were washed in three series of sterile water to remove traces of alcohol. The infected tissue pieces were then suspended in a test tube containing sterilized water for 10 minutes. The bacterial suspension was spread on the surface of TZC medium with spreader. The inoculated plates were incubated at 30°C for 48 hours. The plates were observed for the development of well-separated virulent colonies. It was purified by picking the highly virulent colonies and streaked on the surface of TZC medium contained in Petri dishes. Three to four loopful of well-separated

virulent colonies were suspended in sterile distilled water taken in vials. The vials were stored at 5°C, and served as stock culture for further studies.

Similarly cowpea leaves showing bacterial blight symptom and cabbage leaves with black rot symptom were collected, surface sterilized. The bits were suspended in a drop of sterile water. A loopful of the bacterial suspension was taken on an inoculation needle aseptically and streaked on the surface of solidified nutrient agar medium.

The inoculated plates were incubated at 28°C for 48 hours and were observed for the development of well-separated typical *Xanthomonas* colonies and well grown colonies are selected for purification. Similarly they were stored at 5°C for further studies. The three bacteria isolated from diseased plants were identified on the basis of morphological, cultural and biochemical characteristics prescribed by Bradbury (1986) and Schaad and Stall (1998).

Method of Extraction

Two most common methods used in extraction were followed to extract the antimicrobial components contained in four different plant species in order to screen for their antimicrobial property against the most common and serious phytopathogenic bacteria were (1) Water extract method (2) Alcohol extract method.

Protocol for water extract

The shoot and leaves constituting economic parts of the plants noted in table 1 were used for the purpose of extraction. 50g of leaves or seed as the case may be were taken and cut into small pieces under aseptic condition. The sample was put into waring blender containing 50ml sterilized distilled water at a ratio 1:1 (water: plant material). The sample was spun at low speed for 10-15 minutes in a coffee warring blender till the material formed to fine texture. The blended material was then squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatman no 1 filter paper followed by sterilized Seitz filter. The sterilized filtrate was collected in sterilized glass tubes and the tubes were sealed under aseptic condition and

labelled as “WE”. The water extract was kept at 5°C in a refrigerator for further use.

Protocol for alcohol extract

Fifty gram of the economic parts of the respective plant was mixed with a small quantity of 70 per cent ethyl alcohol and macerated in a pestle and mortar under aseptic condition. The material was blend to fine texture, transferred to a beaker and the final volume was made up to 50ml with 70 per cent ethyl alcohol in the ration of 1:1 (plant material: alcohol). The beaker was kept overnight under refrigerated condition. Alcohol extract was squeezed through muslin cloth, then passed and finally sterilized through Seitz filter apparatus. The sterilized filterate was collected in sterilized glass tubes and the tubes were sealed under aseptic condition and labelled as “AE”. The alcoholic extract was stored at 5°C in a refrigerator for further use.

In-vitro evaluation of plant extracts

Both water and alcohol extracts of the four medicinal plants were screened at different dilutions viz., 1:0 (undiluted), 1:1, 1:10, 1:100, 1:1000. The efficacy of the extracts were tested by the zone of inhibition assay technique against the following bacterial plant pathogens known to cause serious diseases of vegetable crops viz., *Ralstonia solanacearum* causing bacterial wilt of tomato, *Xanthomonas axonopodispv. vignicola* causing bacterial blight of cowpea and *Xanthomonas campestris pv. campestris* causing black rot of cabbage.

Inhibition zone technique

The effect of extracts obtained from economic parts of different plants was tested by inhibition zone technique at different dilutions of the extract. A heavy suspension of the test bacteria (7×10^8 cfu/ml) was seeded to the sterilized nutrient agar medium by mixing the bacterial cultural with the cooled nutrient agar (45-50°C) in a 500ml Erylenmeyer flask. The seeded medium was poured on sterilized Petri plates and allowed to solidify.

Sterilized filter paper disc (Whatman no.1) measuring 10mm diameter were soaked for 10

minutes in undiluted (1:10) and diluted (1:1, 1:10, 1:100 and 1:1000) plant extracts and placed on the surface of seeded nutrient agar medium contained in the Petri plates in marked position. The inoculated plates were incubated first at 4°C for 4 hours so as to allow the diffusion of the extract into the medium. The plates were then transferred to incubator maintained at 30°C and incubated for 48 hours. Observations were recorded on the zone of inhibition produced around the filter paper disc in each plant extract at different dilutions, by measuring the diameter of the inhibition zone.

Results and Discussion

Effect of *Ocimum gratissimum* extract

The results of the experiments conducted on efficacy of water and alcohol extracts obtained from the four medicinal plants were investigated for their effect on the growth of *R. solanacearum* causing bacterial wilt of tomato, *X. axonopodis* pv. *vignicola* and *X. campestris* pv. *camperstris* causing bacterial blight of cowpea by inhibition zone assay technique under *in-vitro* conditions. Water extract of *O. gratissimum* produced inhibition zones of 28.66, 26.66 and 22.66mm at 1:10, 1:1 and 1:10 dilutions respectively but could not produce any zone at 1:100 and 1:1000 dilution against *R. solanacearum*, whereas, alcohol extract of *O. gratissimum* produced inhibition zones of 29.66, 28.33, and 19.33 at 1:1, 1:10 and 1:100 dilution respectively but no inhibition zone was produced at 1:1000 dilution. The water extract of *O. gratissimum* against the *X. axonopodis* pv. *vignicola* induced the inhibition of 28.33, 25.00 and 21.66mm at 1:0, 1:1 and 1:10 dilutions respectively but could not inhibit the growth of *X. a.* pv. *vignicola* at 1:100 and 1;1000 dilution, whereas, alcohol extract produced inhibition zones of 30.33, 28.66, and 20.33 at 1:0, 1:1, 1:10 and 1:100 dilutions respectively but it could not produce any inhibition zone at 1:1000 dilution (Table 2). Further, effect on *X. campestris* pv. *campestris* water extract of *O. gratissimum* produced inhibition zones of 29.33, 24.33 and 22.33mm at 1:0, 1:1 and 1:10 dilutions respectively but could produce any zones at 1:100 and 1:1000 dilution against *X. campestris* pv. *campestris*. Whereas alcohol extract produced inhibition zones. Both the water and alcohol extract of *Ocimum gratissimum* was found to be effective in

inhibiting the growth of three phytopathogenic bacteria tested. Similar inhibitory activity of *O. gratissimum* extract was observed by Rodriguez *et al.*, (1997), Saha *et al.*, (2013) and Mishra *et al.*, (2015) against few bacteria and fungi tested.

Effect of *Ocimum sanctum* extract

In the present investigation it was found that alcohol extract of *Ocimum sanctum* was potent in inhibiting the growth of all the three phytopathogenic bacteria viz., *Ralstonia solanacearum*, *Xanthomonas axonopodis* pv. *vignicola* and *X. campestris* pv. *campestris* upto dilution of 1:10. However, the extract was found to possess highly significant activity at 1:1 dilution and produced an inhibition zone of 20.3, 18.33 and 19.33mm respectively as compared to 14.33, 14.00 and 15.66mm inhibition zone noticed in streptomycin at 400ppm which is used as standard check (Table 3). Even at dilution of 1:10 a significant inhibitory zone in all the three bacterial pathogen was noticed.

However, the water extract was not at all inhibitory as no inhibition zone was obtained against all the three bacteria probably the antimicrobial component eugenol, caryophyllene and methyleugenol might not have been eluded. Rajendhran *et al.*, (1998) observed that extract of *O. sanctum* was very effective against both Gram-positive and Gram-negative bacteria viz: *Staphylococcus aureus*, *E. Coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp. Wood *et al.*, (1997), Citoglu *et al.*, (1998) and Saha *et al.*, (2013) reported inhibitory effect of medicinal plant extracts against Gram-positive and Gram-negative bacteria and few plant pathogenic fungi. Solvent extracts of *Ocimum sanctum* showed inhibited the growth of *R. solanacearum* (Murthy *et al.*, 2014).

Effect of *Tylophora asthmatica* extract

Water extract of another medicinal plant *T. asthmatica* produced an inhibition zone of 17.33 and 13.33mm diameter at 1:0 and 1:1 dilution respectively but could not produce any zones at higher dilution viz, 1:10, 1:100 and 1:1000 against *R. solanacearum*. Whereas, alcohol extract produced inhibition zones of 19.33, 15.66 and 12.33mm diameter at 1:0, 1:1 and 1:10 dilutions respectively but not at 1:100 and 1:1000 dilutions. Streptomycin at 400ppm produced an inhibition zone of 14.33mm diameter.

Water extract of *Tylophora asthmatica* on *X. axonopodis* pv. *vignicola* produced an inhibition zones of 18.33 and 14.33mm diameter at 1:0 and 1:1 dilution respectively but could not produce any zones at higher dilution viz, 1:10, 1:100 and 1:1000 against *X. axonopodis* pv. *vignicola*, whereas, alcohol extract produced inhibition zones of 19.66, 16.66 and 13.33mm diameter at 1:0, 1:1 and 1:10 dilutions respectively, but no inhibition zone was observed at 1:100 and 1:1000 dilutions (Table 4). Streptomycin at 400ppm used as standard check produced an inhibition zone of 14.00mm diameter.

Water extract of *T. asthmatica* on *X. campestris* pv. *campestris* produced an inhibition zones of 24.33 and 23.00 and 18.66mm diameter at 1:0, 1:1 and 1:10 dilution respectively but not at higher dilution of 1:100 and 1:1000 against *X. campestris* pv. *campestris*, whereas, alcohol extract produced an inhibition zones of 25.33, 23.33 and 19.66mm at 1:0, 1:1, and 1:10 dilutions respectively but could not produce any zones at 1:100 and 1:1000 dilutions. While, streptomycin at 400ppm produced an inhibition zone of 15.66mm diameter.

Table.1 List of medicinal plants containing antimicrobial constituents and economic parts used for screening against phytopathogenic bacteria

Sl.No	Common Name	Scientific Name	Family	Parts used
1	Holy basil	<i>Ocimum sanctum</i> L.	Lamiaceae	Leaves
2	Cloves	<i>Ocimum gratissimum</i> L.	Lamiaceae	Leaves
3	Antamul	<i>Tylophora asthmatic</i> W.& A.	Asclepiadaceae	Leaves
4	Garden Rue	<i>Ruta graveolens</i> L.	Rutaceae	Shoot

Table.2 Effect of *Ocimum gratissimum* extracts against three phytopathogenic bacteria

Diameter of inhibition zone (mm)						
Water extract			Alcohol extract			
Dilution	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>
1:0	28.66(5.45)	28.33(5.42)	29.33(5.51)	29.66(5.54)	30.33(5.60)	31.66(5.71)
1:1	26.66(5.26)	25.00(5.10)	24.33(5.03)	28.33(5.42)	28.66(5.45)	26.33(5.23)
1:10	22.66(4.86)	21.66(4.76)	22.33(4.83)	24.33(5.03)	25.66(5.16)	22.00(4.79)
1:100	0.00(1.00)	0.00(1.00)	0.00(1.00)	19.33(4.51)	20.33(4.62)	18.66(4.43)
1:1000	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Streptocycliv 400 ppm. (control)	14.33(3.92)	14.00(3.87)	15.66(4.08)	14.33(3.92)	14.00(3.87)	15.66(4.08)
			S.Em+		CD (1%)	
Factor A			0.0197		0.0556	
Factor B			0.0197		0.0556	
A x B			0.0483		0.1362	

*Figures in parenthesis are square root transformed values

Table.3 Effect of *Ocimum sanctum* extracts against three phytopathogenic bacteria

Diameter of inhibition zone (mm)						
Water extract			Alcohol extract			
Dilution	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>
1:0	0.00(1.00)	0.00(1.00)	0.00(1.00)	23.66(4.97)	20.33(4.62)	21.33(4.72)
1:1	0.00(1.00)	0.00(1.00)	0.00(1.00)	20.33(4.62)	18.33(4.40)	19.33(4.51)
1:10	0.00(1.00)	0.00(1.00)	0.00(1.00)	17.33(4.28)	15.66(4.08)	15.33(4.04)
1:100	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
1:1000	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Streptocycliv 400 ppm. (control)	14.33(3.92)	14.00(3.87)	15.66(4.08)	14.33(3.92)	14.00(3.87)	15.66(4.08)
			S.Em+		CD (1%)	
Factor A			0.0120		0.0338	
Factor B			0.0120		0.0338	
A x B			0.0294		0.0828	

*Figures in parenthesis are square root transformed values

Table.4 Effect of *Tylophora asthmatica* extracts against three phytopathogenic bacteria

Diameter of inhibition zone (mm)						
Water extract			Alcohol extract			
Dilution	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>
1:0	17.33(4.28)	18.33(4.40)	24.33(5.03)	19.33(4.51)	19.66(4.55)	25.33(5.13)
1:1	13.33(3.78)	14.33(3.91)	23.00(4.90)	15.66(4.08)	16.66(4.20)	23.33(4.93)
1:10	0.00(1.00)	0.00(1.00)	18.66(4.43)	12.33(3.65)	13.33(3.78)	19.66(4.55)
1:100	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
1:1000	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Streptocycliv 400 ppm. (control)	14.33(3.92)	14.00(3.87)	15.66(4.08)	14.33(3.92)	14.00(3.87)	15.66(4.08)
			S.Em+		CD (1%)	
Factor A			0.0141		0.0397	
Factor B			0.0141		0.0397	
A x B			0.0345		0.0972	

*Figures in parenthesis are square root transformed values; (control-

Table.5 Effect of *Ruta graveolens* extracts against three phytopathogenic bacteria

Diameter of inhibition zone (mm)						
Water extract				Alcohol extract		
Dilution	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>	<i>R. solanacearum</i>	<i>X. axonopodis</i> pv. <i>vignicola</i>	<i>X. campestris</i> pv. <i>campestris</i>
1:0	0.00(1.00)	0.00(1.00)	0.00(1.00)	16.66(4.20)	14.33(3.91)	15.00(4.00)
1:1	0.00(1.00)	0.00(1.00)	0.00(1.00)	15.00(4.00)	12.33(3.65)	12.00(3.61)
1:10	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
1:100	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
1:1000	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
Streptocycline 400 ppm. (control)	14.33(3.92)	14.00(3.87)	15.66(4.08)	14.33(3.92)	14.00(3.87)	15.66(4.08)
		S.Em+		CD (1%)		
Factor A		0.0091		0.0257		
Factor B		0.0091		0.0257		
A x B		0.0224		0.0630		

*Figures in parenthesis are square root transformed values

The water and alcohol extracts of *Tylophora asthmatica* were found to be effective in inhibiting the growth of all the three phytopathogenic bacteria tested. Similar results regarding inhibitory activity in plant extract of *Cryptostegia grandiflora* belonging to family *Asclepiadaceae* to which *T. asthmatica* also belongs was observed by Mukherjee *et al.*, (1999) against few bacteria viz, *Pseudomonas cepacia*, *Bacillus megatorium*, *B. subtilis*, *B. coagulans*, *Staphylococcus aureus* and *E. coli*. Similarly, Ponnankajamideen *et al.*, (2013) found that *T. asthmatica* extracts obtained from different extracts showed inhibitory effect against different strains of bacteria.

Effect of *Ruta graveolens* extract

The alcohol extract of *Ruta graveolens* root was found to be effective whereas, water extract was ineffective in inhibiting the growth of all the three phytopathogenic bacteria tested. The alcohol extract produced an inhibition zone of 16.66 and 15.00mm diameter at 1:0 and 1:1 dilutions respectively against *R. solanacearum*, but could not produce any zone at 1:10, 1:100 and 1:1000 dilutions. While, streptocycline at 400ppm, produced an inhibition zone of 14.33mm diameter (Table 5).

The alcohol extract effect of *Ruta graveolens* against *X. axonopodis* pv. *vignicola* produced an inhibition zone of 14.33 and 12.33mm diameter at 1:0, 1:1 dilution respectively but could not produce any inhibition zone at higher dilutions of

1:10, 1:100 and 1:1000. Streptocycline at 400ppm produced an inhibition zone of 14.00mm diameter. Finally, the water extract of *Ruta graveolens* was found to be ineffective in inhibiting the growth of *X. campestris* pv. *campestris*. Whereas alcohol extract produced an inhibition zone of 15.00 and 12.00mm diameter at 1:0 and 1:1 dilutions respectively but not at 1:10, 1:100 and 1:1000 dilutions. Streptocycline at 400ppm produced an inhibition zone of 15.66mm diameter. Zobel *et al.*, (1997) found that leaf extract of *R. graveolens* retarded the mitosis activity and they attributed that probably it was due to presence of xanthotoxin and psoralens. The alcohol and water extracts of *R. graveolens* exhibited inhibitory activity against many Gram negative bacterial and plant pathogenic fungi tested (Pandey *et al.*, 2011). The present investigation revealed the antimicrobial nature of the extracts of medicinal plant tested. Hence these could be exploited as an alternate management strategy for chemical pesticides as they are eco-friendly and safe. The future studies should focus on identification and elucidation of the active principles present in medicinal plants having potential antimicrobial properties.

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