Biocidal activity of seed extracts of fruits against soil borne bacterial and fungal plant pathogens

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ABSTRACT

Bacterial and fungal plant diseases affect virtually all crops of economic importance and are difficult to control, resulting devastating financial losses to farmers. The antimicrobial activity of eight fruit seed extract were tested against four plant pathogens *viz*., *Ralstonia solanacearum*, *Xanthomonas campestris*, *Phytophthora capsici* and *Pythium aphanidermatum* by agar well diffusion method. Methanolic extract of *Eugenia jambolana* showed the maximum inhibitory effect against *X.campestris* with the inhibitory zone of 34 mm, whereas the aqueous extract recorded an inhibition zone of 22 mm.

Keywords: Biocide, seed extracts, plant pathogens

INTRODUCTION

Plant diseases are controlled by different fungicides and bactericides, which have vast utilization in the field of agriculture. It has been a subject of public concern due to the harmful effects on the ecosystem and their carcinogenic quality. Hence bioactive compounds from plant extracts can be an alternative against pathogens (Belabid *et al.,* 2010). As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Recio, 1989; Cragg *et al*., 1997). Plant extracts are regarded as constituents in pest management programmes (Belabid *et al.,* 2010). Compared to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have enormous potential against many infectious diseases (Machado *et al.,* 2003; Motsei *et al.,* 2003; Hanil *et al.*,2003; Barbour *et al*.,2004). The bioactive compound of leaf and root extracts of *Carica papaya* was extracted using water and organic solvents and were investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method (Anibijuwon and Udeze, 2009).

The present investigation was carried out to determine the biocidal activity of seed extracts from eight different fruits against more common bacterial and fungal plant pathogens *viz., Ralstonia* sp., *Xanthomonas* sp., *Phytophthora* sp. and *Pythium* sp. This was to identify ecofriendly antagonists from seeds that can serve as deterrants towards pathogenic organisms.

MATERIALS AND METHODS

Preparation of seed extracts

The seeds of the fruits used for the study were *Annona squamosa* (custard apple), *Achras zapota* (chiku or chickle gum), *Carica papaya* (papaya), *Citrullus colocynthis* (water melon), *Eugenia jambolana* (Jamun), *Punica granatum* (pomegranate), *Tamarindus indica* (tamarind) and *Vitis vinifera* (grapes). Seeds of the selected fruits were ground to a fine powder, sieved and dried in hot air oven at 40^0 C. Dry seeds (10g) were powdered, added to 100 mL of methanol or distilled water in conical flask for getting methanolic and aqueous extracts respectively. The preparation was kept in rotary shaker for 72 hrs at 27^0C. The respective extracts were obtained by filtration and subsequently evaporated using Soxhlet apparatus and preserved in screw capped bottles (Akinpelu *et al.,* 2008).

Antimicrobial bioassay

The *in vitro* biocidal activity of seed extracts were investigated against the plant pathogens by 'Agar Well Diffusion Method'. The cultures were swabbed on Modified Nutrient Glucose Agar (MNGA) plates. Wells were bored using sterile cork borer in these plates and 100 µL of respective extracts were loaded and incubated at 37^0C for 24 hrs for bacteria. Fungal plates were incubated at 27^0C for 48-72 hrs. The biocidal spectrum was determined by measuring the zone of inhibition (Akinpelu *et al.,* 2008).

The data was analyzed using the completely randomdamized design with the help of the computer (Microvex system VAX/ VMS, version 5.4, Digital equipment Corporation, University

of Agricultural Science, GKVK, Bangalore. India). The means were compared by Duncan's Multiple Range Test (DMRT) at 5% level.

RESULTS AND DISCUSSION

Ethanol extract of *Syzygium cumini* leaves and aqueous extracts of seeds were observed for the presence of many metabolites namely phenols, falvonoids, tannins, carbohydrates. Both the samples showed the most promising antimicrobial properties against *Clostridium acetobutylicum, Staphylococcus epidermidis* and *Enterococcus faecium* indicating the potential for discovery of antibacterial principles (Shylaja Prabhakaran *et al.*, 2011). Bactericidal results revealed that methanol and aqueous extracts of *E. jambolana* were highly effective ($P<0.05$) against *R. solanacearum* and *X. campestris* (Table 1). Though aqueous extracts of plant extracts did not showed microbicidal activity against *P. aphanidermatum*, both *C. papaya* and *V. vinifens* equally effective against *P. capsici* and *P. aphanidermatum* (Table 2). However, *P. papaya* methanol extract was significantly ($P<0.05$) effective against *P. capsici.* Practitioners usually make use of water as a solvent, but the results observed suggest that methanolic extracts of seeds used in this investigation were much better, powerful and possess antibacterial property. This could be because of better solubility of the active constituents in organic solvents. The seed extracts of *E. jambolana* can be said to have a broad spectrum of antibacterial activity and *C. papaya* showed antifungal activity. Similar observation with methanolic extracts were reported earlier regarding antibacterial activity of *E. jambolana* against two strains of *Staphylococcus aureus* (Recio, 1989). The acetone and methanolic extract of *Manilkara zapota, Annona squamosa* and *Tamarindus indica* seeds were screened against both gram positive and gram negative organisms. Among the three, methanol extract of *T.indica* and acetone extract of *M. zapota* seeds were found to be bactericidal. Similar results were observed with seed extract of *T. indica* in the present investigation.

Aqueous seed extract of *C. papaya* was found to suppress the growth of fungal mycelia of *Rhizopus* sp., *Mucor* sp. and *Aspergillus* sp. This report strengthens the present observation regarding the effectiveness of *C. papaya* seed extract against *P. aphanidermatum* and *P. capsici.* Effect of extracts from *C. papaya* (seed and papain) on mycelial reduction of fungal pathogen causing pawpaw fruit rot has been investigated earlier. Different fungi isolated were *Rhizopus spp, Aspergillus spp* and *Mucor spp* (Nwinyi *et al.*, 2010).

It was also observed that different components that are extractable from the seeds have different levels of inhibition against the growth of bacterial and fungal pathogens. The results of the present investigation support the usage of these fruit seeds especially *E. jambolana* and *C.papaya* as biocides. The most active extracts can be subjected to the isolation of the antimicrobials in order to to carry out evaluation on soil borne plant pathogens. Such studies will lead to ecofriendly measures for protecting crop plants against pathogens.

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Table 1. Biocidal effect of aqueous and methanolic extracts of fruit seeds against *R. solanacearum* and *X. campestris In vitro*.

Extracts	Zone of Inhibition in Diameter (mm)			
	R. solanacearum		*X. campestris*	
	Methanolic Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract
E. jambolana	26 [a]	17 [a]	34 [a]	22 [a]
T. indica	0 [c]	9 [b]	0 [c]	11 [c]
A. zapota	10 [b]	8 [b]	12 [b]	0 [e]
A. squamosa	0 [c]	0 [c]	13 [b]	0 [e]
C. papaya	0 [c]	19 [a]	0 [b]	19 [b]
P. granatum	11 [b]	9 [b]	13 [b]	7 [d]
V. vinifera	0 [c]	0 [c]	0 [c]	0 [e]
C. colocynthis	0 [c]	0 [c]	0 [c]	0 [e]

Means with same superscript are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level.

Table 2. Biocidal effect of aqueous and methanolic extracts of fruit seeds against *P.capsici* and *P.aphanidermatum in vitro.*

Extracts	Zone of Inhibition in Diameter (mm)			
	P. capsici		*P. aphanidermatum*	
	Methanolic Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract
E. jambolana	0 ^{d}	0 ^{c}	0 ^{c}	-
T. indica	0 ^{d}	0 ^{c}	4 ^{bc}	-
A. zapota	10 ^{ab}	7 ^{b}	7 ^{ab}	-
A. squamosa	9 ^{abc}	4 ^{b}	0 ^{c}	-
C. papaya	15 ^{a}	10 ^{a}	12 ^{a}	-
P. granatum	6 ^{bcd}	0 ^{c}	4 ^{bc}	-
V. vinifera	3 ^{bcd}	10 ^{a}	12 ^{a}	-
C. colocynthis	2 ^{cd}	0 ^{c}	5 ^{bc}	-

Means with same alphabets are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level.

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Received: October 09, 2011 Revised: January 24, 2012 Accepted: March 13, 2012