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Plant Latex: A Promising Antifungal Agent for Post Harvest Disease Control

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Abstract: Bioactive compounds from plant latex are potential source of antifungic against post harvest pathogens. Latex from a total of seven plant species was investigated for its phytochemical and antifungal properties. Six fungi namely *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *F. solani*, *P. digitatum* and *R. arrhizus* were isolated from infected fruits and vegetables and tested against various solvent extracts of latex. Analysis of latex extracts with phytochemical tests showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids. Antifungal assay revealed the potential inhibitory activity of petroleum ether extracts against the postharvest fungal isolates. Various degree of sensitivity was observed irrespective of plant species studied with *A. terreus* and *P. digitatum* as the most susceptible ones. *F. solani* and *A. fumigatus* were moderately sensitive to the latex extracts tested. Among the plants, latex of *Thevetia peruviana* (75.2%) and *Artocarpus heterophyllus* (64.8%) were having potential antifungal activity against the isolates followed by *Manilkara zapota* (51.1%). In conclusion, use of plant latex makes interest to control postharvest fungal diseases and is fitting well with the concept of safety for human health and environment.

Key words: Plant latex, postharvest, antifungal, biological control, petroleum ether

INTRODUCTION

Post harvest decays caused by latent or wound induced fungal infections accounts as great as 25-50% loss (Wilson *et al*., 1991). It can occur in the field, during harvesting and subsequent handling and storage. Fruits and vegetables are exposed to a variety of microorganisms starting from field to consumer and fungi are the major group of organisms causing diseases in fruits and vegetables (Chang *et al*., 2008). During ripening and senescence they are more prone to infection (Cantu *et al*., 2009). Reasonable reduction of postharvest decays would minimize the pressure on production resources and generate more revenue in developing countries. Though synthetic fungicides are highly effective their application gets restricted due to pathogen resistance development, non-target specific and accumulation in food chain (Lee *et al*., 2008; Heydari *et al*., 1997). Further, growing concern of health and environment over pesticide residues on fresh commodities have generated growing interest in developing of safer alternatives. Biological control is suitable for postharvest phase of fruits and vegetables (Mari and Guizzardi, 1998). Natural plant products contain antimicrobial activities with low toxicity and broad spectrum activity (Valiathan, 1998, Silver and Bostian, 1990). Plant secondary metabolites have a potential to control postharvest diseases in fruits and vegetables (Neri *et al*., 2009).

About 10% of flowering plants produce latex and are found in over 40 plant families including Euphorbiaceae, Moraceae, Cannabinaceae, Apocynaceae, Astereacea, Papavereceae, Sapotaceae and Asclepidiaceae (Agrawal and Konno, 2009). Latex is an emulsion or suspension of many solid particles and has a clear or variously colored often milky appearance (Dickison, 2000) which exudes upon plant tissue damage (Lewinsohn, 1991; Hunter, 1994; Trease and Evans, 1976). Latex flows inside laticifers of roots, stems, leaves and fruits (Pickyard, 2008). Sub cellular organelles in plant laticifer cells synthesize great varieties of defense proteins and secondary metabolites which are acting as a barrier to microorganisms (Konno, 2011; Ramos *et al*., 2007; El-Moussaoui *et al*., 2001). Latexes contains various biologically active compounds and antimicrobial activities of plant latex has been well documented (Siritapetawee *et al*., 2012; Kanokwiroon *et al*., 2008; Dubey and Jagannadham, 2003; Wititsuwannakul *et al*., 2002; Adikaram *et al*., 1996; Van Parijs *et al*., 1991).

Reducing the postharvest diseases through biological means for the safety of human health and environment is much needed. This study was carried out to investigate the phytochemical and antifungal property

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of the plant latex extracts obtained by varying polarity solvents against the post harvest fungal pathogens of fruits and vegetables.

MATERIALS AND METHODS

This study was conducted during November 2012 to March 2013 and focused on investigating phytochemical and antifungal properties of plant latex against postharvest fungal pathogens.

Plant species: *Artocarpus heterophyllus* Lamb. (Moraceae), *Carica papaya* L. (Caricaceae), *Ficus religiosa* L. (Moraceae), *Manilkara zapota* L. (Sapotaceae), *Jatropha carcus* L. (Euphorbiaceae), *Nerium oleander* L. (Apocynaceae) and *Thevetia peruviana* (Pers.) K. Shum., (Apocynaceae) were selected for the study.

Collection latex: Latex samples were collected from each plant by nipping the leaves near the stem or by incision of the trunk and branches of the plant and allowing the liquid exudates to drain and stored in refrigerator. Based on the type of latex, it was filtered through muslin cloth as well as oven dried.

Extraction of latex: The latex samples were extracted with solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous) for 48 h at room temperature and concentrated to dryness under reduced conditions. The extracts were used for phytochemical (Trease and Evans, 2002; Harborne, 1998; Sofowora, 1993) and antifungal studies.

Isolation of fungal pathogens: Fungal isolation from fruits and vegetables was done by collecting infected fruits from local market and the fungus was inoculated into Potato Dextrose Agar (PDA) plates with chloramphenicol and incubated at 28±1°C for 5-7 days. The colonies were identified (Alexopoulos *et al*., 2007; Naqvi, 2004) and pure culture was maintained in Sabouraud's Dextrose Agar (SDA) slants.

Antifungal assay: Agar well diffusion method was followed to determine the effect of latex extracts against the post harvest fungal pathogens by using Sabouraud's Dextrose Agar (SDA). Spore suspensions of seven day old fungal cultures were prepared in 0.5% tween 80 in sterile distilled water and adjusted to give a spore count of 10^6 spore mL^{-1} using haemocytometer. A 100 µL of the spore suspension was swabbed on the surface of SDA plates and 5 mm well was created on the agar plates. Twenty microliter of the extracts were added to the well and the plates were kept undisturbed for 30 min for the pre-diffusion of the extracts. The plates were incubated at 28°C for 3-5 days and the zone of inhibition was measured using Himedia scale.

RESULTS

Phytochemical analysis of various solvent extracts of latex is represented in Table 1. Alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids were widely distributed in most of the extracts tested. A total of six fungal strains viz., *Aspergillus fumigatus, A. niger, A. terreus, Fusarium solani, Penicillium digitatum* and *Rhizopus arrhizus* were isolated from infected fruits and vegetables (Fig. 1).

Antifungal activity of the solvent extracts of latexes is represented in Table 2. Petroleum ether extracts of latexes have exhibited stronger and consistent antifungal activity against the most of the isolates tested. Percent inhibition of petroleum ether latex extract is represented in Fig. 1. Aqueous extracts were completely failed to inhibit the growth of test fungi throughout the study. *A. heterophyllus* has exhibited significant antifungal activity (≥3.52 mm) with complete inhibition of *A. terreus* and *P. digitatum*. Methanolic extract showed moderate activity against all the three species of *Aspergillus* (7.4 to 10.3 mm) and acetone extract recorded inhibitory activity against *A. terreus* (8.8 mm). Various degree of inhibition (7.5 to 45.3 mm) was observed with petroleum ether extract of *C. papaya* latex with no activity against *A. fumigatus*. Methanolic extract also exhibited inhibitory activity against three of the total fungi tested. *F. religiosa* was effective against all the isolates (16.89 to 45.7 mm) and the other solvent exhibited antifungal activity apart from petroleum ether was methanolic extract against *A. niger* (20.2 mm). *A. terreus* and *F. solani* were the most sensitive organisms which were completely inhibited by petroleum ether extract of *M. zapota* throughout the incubation period. *P. digitatum* was the other fungi controlled by its latex (47 mm).

Moderate activity was recorded with petroleum ether extract of *J. carcus* (11.5 to 34.0 mm) and *N. oleander* (10.6 to 25.3 mm) latex. Ethyl acetate and acetone extracts of *Jatropha* latex has demonstrated antifungal activity against *F. solani* (5 mm), *A. fumigatus* (2.5 mm) and *A. terreus* (7.5 mm). Sensitivity of *R. arrhizus* was observed against ethyl acetate extract of *N. oleander*.

Table 1: Phytochemical analysis of solvent extracts of plant latex

Plant	Sol	Alk	Fla	Gly	Phe	Sap	Ste	Tan	Ter
A. heterophyllus	Pet	-	+	-	-	-	-	-	+
	Chl	-	-	+	-	-	-	-	+
	Eth	-	-	+	-	-	-	-	+
	Ace	-	-	+	-	-	-	-	-
	Met	-	-	+	-	-	-	-	-
	Aqu	-	-	+	-	+	+	-	-
C. papaya	Pet	+	-	+	-	+	-	-	+
	Chl	+	-	+	-	+	-	-	+
	Eth	+	-	+	-	-	-	-	-
	Ace	+	-	+	-	-	-	-	+
	Met	+	-	-	-	-	-	-	-
	Aqu	-	-	+	-	+	-	-	-
F. religiosa	Pet	-	+	-	-	+	-	-	+
	Chl	-	-	-	-	+	-	-	+
	Eth	-	+	+	-	-	-	+	+
	Ace	+	-	+	+	+	-	+	-
	Met	+	-	-	+	-	-	+	-
	Aqu	+	+	-	+	-	-	+	-
M. zapota	Pet	-	+	+	-	+	-	-	+
	Chl	-	-	-	-	-	-	-	+
	Eth	-	-	+	-	+	-	-	+
	Ace	+	+	+	-	-	-	-	+
	Met	+	-	+	-	-	-	-	+
	Aqu	+	-	+	-	+	-	-	+
J. carcus	Pet	+	-	-	-	-	-	-	-
	Chl	-	-	-	-	-	-	-	-
	Eth	-	-	-	-	-	-	-	-
	Ace	+	-	-	+	+	+	+	+
	Met	+	-	-	+	+	+	+	+
	Aqu	-	-	-	-	+	-	-	+
N. oleander	Pet	-	-	-	-	-	-	-	-
	Chl	-	-	-	-	-	-	-	+
	Eth	-	-	+	-	-	-	-	-
	Ace	-	-	+	-	-	-	+	-
	Met	-	-	-	-	+	-	+	-
	Aqu	+	-	-	-	+	-	-	+
T. peruviana	Pet	-	+	-	-	-	-	-	+
	Chl	-	-	-	-	-	-	-	+
	Eth	-	+	-	-	-	-	-	+
	Ace	-	-	+	-	-	-	-	+
	Met	+	-	+	-	-	-	-	-
	Aqu	-	-	-	-	+	-	+	-

Alk: Alkaloids, Fla: Flavonoids, Gly: Glycosides, Phe: Phenols, Sap: Saponins, Ste: Steroids, Tan: Tannins, Ter: Terpenoids, Pet: Petroleum ether, Chl:Chloroform, Eth: Ethylacetate, Ace: Acetone, Met: Methanol, Aqu: Aqueous, +: Presence; -: Absence

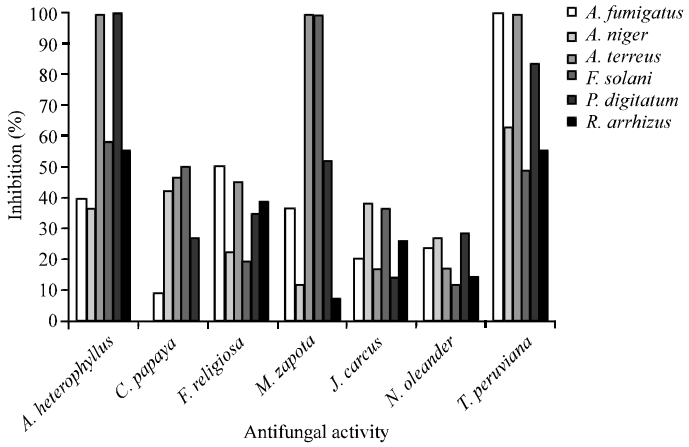

Fig. 1: Percent inhibition of petroleum ether latex extract

Maximum antifungal activity (76 mm) of petroleum ether extract of *T. peruviana* was demonstrated against all the fungal pathogens with complete inhibition of *A. fumigatus* and *A. terreus*. Ethyl acetate was the other solvent extract

Table 2: Zone diameter of inhibition of plant latex (mm)

Plant	Sol	*A. fumigatus*	*A. niger*	*A. terreus*	*F. solani*	*P. digitatum*	*R. arrhizus*
A. heterophyllus	Pet	35.5±0.6	32.5±0.1	NG	52.5±0.0	NG	50.0±0.8
	Chl	NA	NA	NA	NA	NA	NA
	Eth	NA	NA	NA	NA	NA	NA
	Ace	NA	NA	8.8±0.2	NA	NA	NA
	Met	10.3±0.5	7.4±0.7	10.1±0.7	NA	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA
C. papaya	Pet	NA	7.5±0.7	37.5±0.1	41.7±0.8	45.3±0.0	23.5±0.2
	Chl	NA	NA	NA	5.0±0.	NA	NA
	Eth	NA	NA	NA	NA	NA	NA
	Ace	NA	NA	NA	NA	NA	NA
	Met	NA	3.9±0.	8.7±0.	27.4±0.	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA
F. religiosa	Pet	45.7±0.2	20.1±0.1	40.0±0.3	16.8±0.8	30.0±0.5	34.6±0.7
	Chl	NA	NA	NA	NA	NA	NA
	Eth	NA	NA	NA	NA	NA	NA
	Ace	NA	NA	NA	NA	NA	NA
	Met	NA	20.2±0.9	NA	NA	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA
M. zapota	Pet	32.7±0.2	10.1±0.2	NG	NG	47.2±0.2	6.1±0.2
	Chl	NA	NA	NA	NA	NA	NA
	Eth	NA	NA	NA	NA	NA	NA
	Ace	NA	NA	NA	NA	NA	NA
	Met	NA	NA	NA	NA	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA
J. carcus	Pet	17.5±0.1	34.0± 0.8	15.0±0.2	32.5±0.2	11.5±0.0	22.5±0.4
	Chl	NA	NA	NA	NA	NA	NA
	Eth	NA	NA	NA	5.0	NA	NA
	Ace	2.5±0.	NA	7.5±0.	NA	NA	NA
	Met	NA	NA	NA	NA	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA
N. oleander	Pet	20.7±0.8	23.6±0.7	15.2±0.6	10.6±0.0	25.3±0.7	12.1±0.9
	Chl	NA	NA	NA	NA	NA	NA
	Eth	NA	NA	NA	NA	NA	10.9±0.6
	Ace	NA	NA	NA	NA	NA	NA
	Met	NA	5.0	NA	NA	NA	10.0
	Aqu	NA	NA	NA	NA	NA	NA
T. peruviana	Pet	NG	56.7±0.1	NG	44.2±0.4	76.0±0.7	49.5±0.6
	Chl	NA	NA	NA	NA	NA	NA
	Eth	35.4±0.0	7.1±0.6	50.7±0.7	22.3±0.9	30.7±0.1	20.8±0.0
	Ace	NA	NA	10	NA	NA	NA
	Met	NA	NA	NA	NA	NA	NA
	Aqu	NA	NA	NA	NA	NA	NA

NA: No activity

of *T. peruviana* latex which has exhibited inhibitory against all the isolates. However, other solvent extracts were failed to control the growth of fungal pathogens tested.

DISCUSSION

Postharvest decay loss of fruits and vegetables can range from 10-50% depending on the commodity and country (El-Ghaouth *et al*., 2004) and serious losses of horticultural produce result from postharvest disease development. In spite of the advantages brought by synthetic fungicides, excessive use of it has taken its toll both on environment and human health. There is a worldwide trend to explore safe alternatives to control post harvest diseases and plant pathologists are interested in natural compounds derived from plants (Johnson and Sangchote, 1994). Many promising potential alternatives which include naturally occurring antifungal compounds, microbial antagonists and induced resistance have been developed for postharvest decay control.

Antimicrobial activities of plant latexes have been well documented (Guerrero and Guzman, 2004). Trindade *et al.* (2006) has characterized chitin binding lectins from *Artocarpus* and found its antifungal activity against *Fusarium moniliforme*. Potential anti mycotic activity against postharvest fungal pathogens by *A. heterophyllus* latex was seen throughout this investigation. Potential antifungal activity of Papaya seeds and latex were studied earlier (Quintal *et al*., 2011; Giordani *et al*., 1996). A significant inhibitory action against *F. solani* (41.7 mm) and *P. digitatum* (45.3 mm) were exhibited by *C. papaya* latex from our findings.

Antifungal activity of *F. religiosa* leaves were reported by previous studies (Aqil and Ahmad, 2003; Hemaiswarya *et al*., 2009) and a significant inhibitory action of its latex was documented in the present findings. *M. zapota* is documented for its antimicrobial activities (Kaneria and Chanda, 2012; Nair and Chanda, 2008) and the antifungal activities of its latex were demonstrated in this investigation. Ethanolic extracts of *Jatropha curcas* was reported against phytofungal pathogens (Saetae and Suntornsuk, 2010) and a moderate inhibition (11.5 to 34 mm) was observed from *Jatropha* latex in this study. Hadizadeh *et al.* (2009) has screened *Nerium oleander* for its antimycotic activity and observed its potential activity against plant pathogenic fungi. The present findings revealed the antifungal activity of *N. oleander* latex against plant pathogens. Antimicrobial activities of *Thevetia peruviana* have been reported in previous studies (Patil *et al*., 2007; Gata-Gonclaves *et al*., 2003). In this study, *T. peruviana* latex was observed with potential antifungal activity against all the isolates tested.

In this study, petroleum ether extracts exhibited prominent antifungal activity than the other extracts. Antifungal activity of petroleum ether extracts of various species of *Plumeria* latex has been reported (Sibi *et al*., 2012). Raghavendra and Mahadevan (2011) have reported the antimicrobial activities of petroleum ether extracts of latex of various plants. In the present study, most of the fungi were sensitive to the latex extracts but were able to grow from third day of incubation. This could be due to the presence of volatile compounds or less activity of bioactive compounds during prolonged incubation.

Considering the easy availability of the above studied plants, there are possibilities for scientific studies to fully exploit the medicinal properties of their latex for exploring promising 'leads'. Further, the use of plant latex makes interest to control postharvest fungal diseases and is fitting well with the concept of safety for human health and environment.

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