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Antifungal properties of *Plumeria acutifolia* and their effect on the acid and alkaline phosphatase of the fungal pathogens

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In the present investigation, solvent extract of latex of *Plumeria acutifolia* Poir was screened for its antifungal properties against twelve fungal strains. It showed antifungal properties against four fungi viz. *Acremonium kiliense, Myrothecium roridum, Penicillium expansum* and *Rhizoctonia solani.* The effect of this extract on the acid and alkaline phosphatase enzyme system of this four fungal pathogen was also studied.

Key Words : *Plumeria acutifolia*, acid and alkaline phosphatase, pathogens

INTRODUCTION

Fungal diseases of plants are creating problems throughout the world, and fungicides are used to control such diseases. But indiscriminent uses of these antimicrobial compounds have caused potential threat to human health and cause environmental hazards. These have prompted the scientists to search for safe, eco-friendly, non-toxic and effective antimicrobial agents from the safest possible sources of plants. Screening and reviewing of plant extracts for antimicrobial activities have been carried out by several scientists (Osborn, 1943; Ghosh *et al,* 1980; Thakur *et al,* 1994; Ahmad *et al,* 1998; Cowan, 1999; Yossry *et al,* 1999; Gomathi and Kannabiran, 2000; Nascimento *et al,* 2000; Bagul and Patel, 2001; Ratti *et al*, 2002; Dubey and Kumar, 2003; Prasad and Barnwal, 2004; Sengupta *et al*, 2004; Khurana *et al,* 2005; Saha *et al*, 2005).

In the present investigation attempt has been made to screen the antifungal properties of the latex of *Plumeria acutifolia* Poir. (Apocynaceae) against some pathogenic fungi. and to study the effect of this extract on the acid and alkaline phosphatase enzymes of the pathogens.

MATERIALS AND METHODS

Latex of *Plumeria acutifolia* Poir was collected from the plant and mixed with ethanol in 1:5 ratio and kept it for few hours at room temperature. Then this was slightly warmed for concentrating the volume. The pH of the extract was determined by dipping the high sensitivity pH paper in the extract. The antifungal activities of the plant extracts were tested against *Aspergillus niger* Van Tieghem, *Acremonium kiliense* Gruitz, *Alternaria brassicicola* (Schw.) Wiltshire, *Cladosporium herbarum* (Pers.) Link, *Colletotrichum capsici* (Synd.) Bulter and Bisby, *Curvularia lunata* (Wakker) Boedijn, *Fusarium udum* Bulter, *Myrothecium roridum* Tode ex Fries, *Penicillium expansum* Link ex Fries, *Phytophthora parasitica* Dastur, *Rhizoctonia solani* Kuehn, and *Scrophulariopsis* sp. All these fungal strains were obtained from the stock culture of the Mycology and Plant Pathology Research Laboratory, P. G. Department of Botany, Presidency College, Kolkata, and later maintained on PDA medium in the laboratory.

The antifungal activities of this plant extract were assayed by filter paper disc diffusion method

(Whatman No. 1 and the diameter of paper discs – 6 mm). The fungal inocula were prepared from mycelial or spore suspension. The inocula (10^6/ml) in each case were incorporated (1 ml for fungal strains) in respective agar medium. Sterilized discs were soaked in the plant extract for 5 minutes, hold in air for 1-2 minutes and were then carefully placed in the freshly prepared patriplates seeded with test organisms. Control plates received the solvent only. A multiple copies (3 times) of the prototypes were prepared for each experimental set. The diameter of inhibition zones around the discs was measured after 48–120 hrs at 28°C.

The effect of the extract on enzymes (acid and alkaline phosphatase) and total protein were determined. Microbial media saturated with inocula (72-96 hrs at 28°C for fungi) were harvested by spinning in centrifuge at 3000 rpm for 5 minutes. The pellets were resuspended in respective enzyme buffer (0.1 M acetate buffer with pH 5.2 for acid phosphatase; 0.1 M carbonate-bicarbonate buffer with pH 10 for alkaline phosphatase and phosphate buffer with pH 7.6 for total protein) in an appropriate volume (1:5 for phosphatase and 1:25 for protein) and crushed with a prechilled mortar pestle with an optimum quantity of pH-equilibrated sand. The slurries were centrifuged at 5000 rpm for 10 minutes and the supernatant enzyme solution were again centrifuged at 8000 rpm for 10 minutes. The supernatants (the enzyme sources) were decanted off and pooled together into a tube and stored at 0°C until further use.

The ingredients viz. substrate, respective buffer and microbial enzyme were mixed in appropriate volume (0.5 ml substrate, 1.45 ml respective enzyme buffer and 0.05 ml microbial enzyme) for testing the acid and alkalin phosphatase activity. Here para-nitrophenyl phosphate (5 mmol or 50 µmol/L) was used as substrate. Then the reaction mixtures were incubated for 1 h at 30-35°C. After incubation 0.1 (N) NaOH was added successively and optical density (OD) values were recorded at 410 nm weve length of light keeping the respective buffer as control. To study the effect of antimicrobial substances of selected plant extract on acid and alkaline phosphatase enzymes, the sequence of chemicals added to the reaction mixture were mentioned at before (except respective buffer which is 1 ml here); in addition to them the plant extract (0.45 ml) was added to the reaction mixture. The OD values were recorded at 410 nm after proper incubation. The results thus obtained were calculated in terms of specific activities of phosphates (µg of para-nitrophenol released / mg of protein / h) and the effects of the plant extract on the organisms were studied as antagonistic or synergistic effects. The protein content of each sample was estimated by Lowry method.

The standard curves for acid and alkaline phosphatase (5 mmol or 50 µmol/L para-nitrophenol as standard) and total protein (bovine serum albumin as standard) were prepared according to protocol (Plummer, 1979; Banerjee and Adhikari, 1986; Sadasivam and Manickam, 1996).

RESULTS AND DISCUSSION

From the disc diffusion assay technique, it could be revealed that, the extract of *P. acutifolia* showed huge amount of antifungal activities against *A. kiliense, M. roridum, P. expansum and R. solani.* (Table 1). The pH values of the extract was 6.0 at the time of treatment, which was moderately acidic and this result indicated that the inhibition zones produced were certainly due to active principles present in this extract, not due to the pH.

Table 1 : *In vitro* activities of extracts of *Plumeria acutifolia* Poir against some bacteria and fungi

Name and Plant Parts Used	Solvent Used	Some bacterial and fungal strains (Diameter of inhibition zones including diameter of paper discs - 6 mm)											
		An	Ak	Ab	Cc	Cl	Ch	Fu	Mr	Pe	Pp	Rs	S
Plumeria acutifolia (Latex)	Ethanol	–	18	–	–	–	–	–	22	17	–	22	–

Key : An = *A. niger*; Ak = *A. kiliense*; Ab = *A. brassicicola*; Cc = *C. capsici*; Cl = *C. lunata*; Ch = *C. herbarum*; Fu = *F. udum*; Mr = *M. roridum*; Pe = *P. expansum*; Pp = *P. parasitica*; Rs = *R. solani*; S = *Scrophulariopsis* sp.;
"–" = No inhibition zone

Phosphatases liberate inorganic phosphate from organic phosphate ester. The enzyme phosphatase hydrolysed para-nitrophenyl phosphate and released yellow colored para-nitrophenol in alkaline medium. Acid phosphatase hydrolysed a number of phosphomonoester and phosphoproteins, whereas alkaline phosphatase catalysed the hydrolysis of

numerous phosphate esters. In this study of effects of selected plant extracts viz. *P. acutifolia* on the standard specific activity of acid phosphatase of *A. kiliense*, a prominent increase of overall enzyme activity was noticed. Similar increasing activities had been found in case of *P. acutifolia* against *P. expansum* and *M. roridum*. But in case of standard specific activity of acid phosphatase of *P. acutifolia* on *R. solani*, a decrease of overall enzyme activity was observed. On the other hand, the effect of extract of *P. acutifolia* on the standard specific activity of alkaline activities had been found, whereas against *R. solani*, it showed increase activity. *P. acutifolia* on *M. roridum* did not show any phosphatase activities in alkaline medium (Table 2). The incrase and decrease of overall enzyme activities might have indicated the synergistic and antagonistic interactions in between the plant extracts and test organisms respectively. This type of antimicrobial property was extrapolated with respect to phosphatase activities, which might be due to the direct effect on nucleic acids and some energy riched compounds like ATP of tested fungal pathogens.

Table 2 : Effect of extracts of *Plumeria acutifolia* Poir on standard specific activities of acid and alkaline phosphatase of fungi

Enzyme System	Enzyme Sources (from Pathogen)	Standard Specific Activity (µg/mg/h) (X)	Altered Specific Activity (µg/mg/h) (Y)	Difference in Specific Activity (Y-X)	Percentage in Specific Activity [Y-X/X] × 100
Acid Phosphatase	*A. kiliense*	3.87	15.35	+11.48	+296.64
	M. roridum	1.40	5.80	+4.40	+314.29
	P. expansum	6.96	26.61	+19.65	+282.33
	R. solani	2.58	1.22	–1.36	–52.71
Alkaline Phosphatase	*A. kiliense*	5.51	4.38	–1.13	–20.51
	M. roridum	0.00	0.00	0.00	0.00
	P. expansum	3.31	1.46	–1.85	–55.89
	R. solani	3.67	5.24	+1.57	+42.78

Key : Specific Activity = µg of para-nitrophenol release / mg of protein / h;
Standard Specific Activity = Specific activity of the pathogen (fungi); Altered Specific Activity = Specific activity of the pathogen changes after addition of plant extract.

The activities of the plant extracts against microorganisms might be due to the synergistic effect of the active principles present in the plant extracts. The present study being a screening programme, employed *in vitro* experiments. However, to ascertain the natures of the active principles and their activities *in vivo*, which were also the prerequisites for *in situ* applications, further investigations are in progress to isolate, purify and characterize the active principles from the extracts. Moreover, similar screening programme had also been undertaken with a view to find out potent, broad spectrum and environmentally safe antimicrobial compounds that could replace the toxic and hazardous synthetic compounds.

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