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SHORT COMMUNICATION

ANTIFUNGAL ACTIVITY OF *BARRINGTONIA CEYLANICA* BARK EXTRACT

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Abstract: A 20% methanol in methylene chloride flash column fraction of the extract of the bark of *Barringtonia ceylanica* was found to contain antifungal activity. The extract inhibited the growth of several fungal plant pathogens *Curvularia* sp, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Cylindrocladium quinqueseptatum* and *Rigiodiporus lignosus*. Complete inhibition of growth of *Curvularia* sp and *Colletotrichum gloeosporioides* was observed. The inhibition of growth of *Cylindrocladium quinqueseptatum* and *Rigiodiporus lignosus* was more than 50%.

Key words: Antifungal activity, *Barringtonia ceylanica*, *Colletotrichum gloeosporioides*, *Curvularia* sp., *Cylindrocladium quinqueseptatum*, *Rhizoctonia solani*, *Rigiodiporus lignosus*.

INTRODUCTION

Sri Lanka, with its great diversity of flora and fauna posseses many plants of medicinal value.[1-5] Discovery of metabolites showing biological activity has led to extensive research programmes aimed at the isolation and characterization of biologically active metabolites from Sri Lankan plants. Many extracts of local plants have been screened for antifungal activity and some plant extracts are reported to have significant antifungal activity.[6-10] *Barringtonia ceylanica* belongs to the family Lecythidaceae. *Barringtonia ceylanica* is used in traditional medicine. The bark is reportedly effective against gastric ulcers and together with leaves have also been used for rat and snake bites, rat poisoning and on boils. The leaves have been used for the treatment of dysentery and to arrest bleeding from cuts.[3] No previous study on the antifungal activity of this plant has been reported. Fungicides extracted from locally available natural products can be developed as alternatives for expensive imported fungicides.[8,10] We report here on the antifungal activity of the 20% methanol in methylene chloride flash column fraction of the extract of *Barringtonia ceylanica* against several fungal plant pathogens.

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Trees of the genus *Barringtonia* have been common in the past but well grown trees are now not common.[11] *Barringtonia ceylanica* is a small tree, with a rough gray bark, capable of reaching 20 m or more. It is distributed from Natal in Southern Africa to Kenya, and also in the Western Pacific from Fiji and Samoa to Queensland. It grows in moist low country, on the shores of backwaters, estuaries, lakes and rivers and also inland in similar environment. It flowers all year, coastal trees usually with pinkish filaments, inland trees with crimson filaments. The leaves of inland trees are usually smaller than coastal trees.[11] Our specimen was collected inland from Mulleriyawa.

METHODS AND MATERIALS

Preparation of the bark extract : One kg of *Barringtonia ceylanica* bark was washed under running water and air dried, cut into small pieces, ground in a laboratory mill and kept immersed in methanol(5 l) for 2 weeks. The methanol extract was filtered through cotton wool and solvent removed under reduced pressure at 40-45^{0}C to yield a crude extract (22.5 g). The crude extract was subject to flash chromatography using G-6 silica gel and solvent systems of increasing polarity (hexane, methylene chloride in hexane, methanol in methylene chloride). These fractions were concentrated under reduced pressure at 40-45^{0}C using a rotary evaporator and tested for antifungal activity by *Cladosporium* thin layer chromatography (tlc) bioassay.[12] All solvents were distilled before use.

Cladosporium tlc-bioassay: Test solutions were spotted on t.l.c plates (Aldrich, tlc grade silica pre-coated to a thickness of 0.5mm on 20x20 cm glass plates). Six sets of plates were separately developed in solvent systems of increasing polarity (1:1 hexane:methylene chloride, 3:1 methylene chloride in hexane, methylene chloride, 5% methanol in methylene chloride, 10% methanol in methylene chloride and 20% methanol in methylene chloride) in solvent tank equilibrated with eluant. After air drying at ambient temperature for 24 hours, the plates were sprayed with a conidia suspension and incubated as described by Smith.[12]

The 20% methanol in methylene chloride flash column fraction (containing about 4g of compound) showed antifungal activity against *Cladosporium cladosporioides* and was used to study antifungal activity against other plant pathogens.

Organisms: *Cladosporium cladosporioides*, *Curvularia* sp. isolated from infected leaves of rice plants showing spots, *Colletotrichum gloeosporioides* isolated from infected rubber leaves (*Colletotrichum* leaf disease), *Rhizoctonia solani* isolated from infected sheaths of rice plants (sheath blight disease),

Cylindrocladium quinqueseptatum isolated from infected clove leaves (leaf fall disease) and *Rigiodiporus lignosus* isolated from infected rubber roots (white root disease) were used as test fungi. All fungi were maintained on potato dextrose agar(PDA) at 30°C.

Agar Plate Assay: Fifteen ml portions of sterile molten PDA were cooled to 45°C and were mixed with volumes of test solution (20mg/ml of test sample in acetone) so that the final concentration of test compounds were 0.01%, 0.02%, 0.05% by weight, and then poured into sterile petri dishes. The medium incorporated with the compounds was inoculated with the test fungi. The inoculum, which consisted of a 0.5 cm^2 agar square obtained from the periphery of a 7d old fungal culture growing on PDA at room temperature was placed at the centre of the medium. Three plates were used per test fungus. In the control experiments the medium was prepared using only the corresponding volume of acetone. All plates were incubated at room temperature and growth was measured as described in Senaratna *et al.*[13] at 24 h intervals for 7d; growth on day 4 was used for the calculations. The experiments were carried out in triplicate.

The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = \left[\frac{\text{growth area in reference - growth area in samples}}{\text{growth area in reference}} \right] \times 100$$

RESULTS AND DISCUSSION

The preliminary screening of the flash column fraction for antifungal activity was done by tlc plate bioassay method using *Cladosporium cladosporioides*. Two of the flash column fractions (10% methanol in methylene chloride (2 g residue), 20% methanol in methylene chloride (4 g residue)) showed inhibitory zones in the bioassay. The 20% methanol in methylene chloride fraction was chosen for study of the inhibitory action against plant pathogens because of its higher inhibition and higher weight of residue. The % inhibition of fungal growth by 20% methanol in methylene chloride fraction of *Barringtonia ceylanica* bark extract obtained using agar plate bioassay is given in Table 1.

Isolation and characterization of the secondary metabolites responsible for this inhibitory activity against plant pathogens would enable them to be developed as an inexpensive fungicide based on locally available natural products.

Table 1: Inhibition of fungal growth by 20% methanol in methylene chloride fraction of *Barringtonia ceylanica* bark extract.

Fungus	% inhibition of growth* by test sample at 0.01%	0.02%	0.05%
Curvularia sp .	100 ± 0	100 ± 0	100 ± 0
Cladosporium cladosporioides	93 ± 4[a]	100 ± 0[a]	100 ± 0[a]
Colletotrichum gloeosporioides	100 ± 0	100 ± 0	100 ± 0
Cylindrocladium quinqueseptatum	72 ± 6[b]	66 ± 9[b]	60 ±11[b]
Rigiodiporus lignosus	45 ± 3[c]	50 ± 7[c]	74 ± 2[c]
Rhizoctonia solani	10 ± 7[d]	13 ± 6[d]	9 ± 3[d]

*Average of three replicates ± standard error of the mean. Values in a row followed by the same letter are not significantly different at p = 0.05 (Duncan's multiple ranges test).

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