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ANTIFUNGAL PROPERTIES OF CROTON AROMATICUS, C. LACCIFERUS AND C. OFFICINALIS INCLUDING THE ISOLATION OF A FUNGICIDAL CONSTITUENT

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ABSTRACT

Extractives of Croton aromaticus, C. lacciferus and C. officinalis were tested for antifungal activity against Cladosporium cladosporioides. Steam distillates of the leaves of C. aromaticus and C. lacciferus, and the root extracts of C. officinalis displayed considerable antifungal properties. The root extracts of C. lacciferus were moderately active while those of C. aromaticus were inactive. Six compounds isolated from the active root extracts of C. lacciferus and C. officinalis were examined for antifungal properties. Only 2,6-dimethoxybenzoquinone isolated from the chloroform extract of C. lacciferus roots exhibited activity. Comparatively less quantities of 2,6-dimethoxybenzoquinone were required for the inhibition of the growth of the three plant pathogenic fungi (Botrydiplodia theobromae, Colletotrichum gloeosporioides and Gibberella fujikuroi) than for the inhibition of the growth of the two saprophytes (Aspergillus niger and Cladosporium cladosporioides).

INTRODUCTION

Identification of new sources for antifungal compounds, particularly of plant origin, is useful in the development of prospective fungicides with specific properties. Plant-based fungicides may have less harmful effects on mammals than the synthetic compounds.

Natural products having antifungal properties are known (Siegel and Sisler, 1977). Argyrophillic acid, a constituent of Croton argyrophylloides has, for example, shown antimicrobial properties (de Albuquerque et al., 1974). The present study involves the investigation of the extractives of C. aromaticus L.,C. lacciferus L. and C. officinalis Klotsch, and some root

constituents of the latter two plants for the presence of antifungal activity against Cladosporium cladosporioides. The evaluation of the antifungal properties of the active constituent in the roots of C. lacciferus against five fungi is also described.

MATERIALS AND METHODS

Plant extractives

Plant samples of C. aromaticus, C. lacciferus and C. officinalis were collected near Dambulla, Peradeniya and Puttalam, respectively. Crushed half-dried leaves of C. aromaticus and C. lacciferus were separately subjected to steam distillation. Each distillate was extracted with diethyl ether and dried over anhydrous magnesium sulfate. Concentration of the ethereal solution in a rotavapor yielded the steam distillate as a brown oil for each plant. The dried and powdered roots of C. aromaticus, C.lacciferus and C. officinalis were separately and sequentially extracted with light petroleum (b.p. 60-80^{0}C), chloroform and methanol under reflux conditions. The removal of solvent in a rotavapor gave the three extracts for each plant. The weights of extractives and the corresponding plant material are given in Table 1.

Cladosporium-TLC-bioassay

The root extracts (petroleum, chloroform and methanol) (2 mg each) of the three Croton species and the steam distillates (leaves) (2 mg each) of C. aromaticus and C. lacciferus were spotted on TLC plates (silica gel 60 $PF_{254-366}$, 0.5 mm x 20 cm x 20 cm) and the plates were developed in chloroform. The plates, after air-drying overnight, were sprayed with a suspension of conidia of Cladosporium cladosporioides in Czapek-Dox nutrient solution and incubated in a moist chamber at 26 + 2^{0}C for 48 hours (Klarman et al., 1968). The regions in which the growth was inhibited, appeared light coloured against a background of green mycelia. The diameters of the zones of inhibition were measured in mm; the zones of inhibition were usually approximately circular. Benlate (0.2 mg, in methanol; 50% active ingredient, methyl-1-(butylcarbonyl-2-benzimidiazolecarbamate, Du Pont, USA) was spotted on each TLC plate as the standard and the diameter of the resultant inhibition area was measured.

Antifungal activity of the root constituent of C. lacciferus and C. officinalis

The chloroform extract (30 g) of C. lacciferus roots was chromatographed on a silica gel column and elution with n-hexane-chloroform, followed by medium pressure column chromatography of the major fractions, to give

ent-kaur-15-en-17-ol (56 mg), m.p. 136-137°C, ent-kaur-15-en-17-hydroxy-3β-yl acetate (32 mg), m.p. 130-131°C, ent-kauran-3β,16 , 17-triol (70 mg), m.p. 218-220°C, and 2,6-dimethoxybenzoquinone (19 mg), m.p. 242-245°C. The light petroleum extract (15 g) of C. officinalis roots was stirred with light petroleum (20 ml), and the insoluble residue was recrystallized from diethyl ether to obtain officinalin (1.1 g), m.p. 138-138.5°C. The chloroform extract (20 g) of C. officinalis roots was chromatographed on a silica gel column and elution with light petroleum-dichloromethane, followed by medium pressure column chromatography, to afford penduliflaworosin (42 mg), m.p. 99-100°C.

These six compounds (2 mg each) were spotted on a TLC plate and the plate was subjected to the Cladosporium-bioassay as described above.

Antifungal properties of 2,6-dimethoxybenzoquinone

Antifungal properties of 2,6-dimethoxybenzoquinone was further tsted against Botrydiplodia theobromae, Colletotrichum gloeosporioides, Giberella fujikuroi, Aspergillus niger and Cladosporium cladosporioides using the TLC plates as described above. In the case of C. cladosporioides and A. niger, the regions in which the growth was inhibited, appeared light coloured against green mycelia background. Inhibition regions for the other three fungi (B. theobromae, C. gloeosporioides and G. fujikuroi) were detected after standing the mycelium by dipping the plates in a saturated iodine bath. Inhibition areas corresponding to varying amounts of 2,6-dimethoxybenzoquinone were measured using a fixed arm planimeter.

RESULTS AND DISCUSSION

The results of screening the extractives of the three Croton species for antifungal activity against C. cladosporioides are given in Table 1. The steam distillates of the leaves of C. aromaticus and C. lacciferus have displayed significant activity. Both distillates appear to contain the same active principles at R_f 0.4 and 0.6 in comparable quantities. The compound corresponding to R_f 0.4 was presumably either more active or more abundant in the distillate than that at R_f 0.6. The steam distillate of C. aromaticus contains an active compound of lower polarity, R_f 0.2, which is absent in the leaves of C. lacciferus. Two active components (R_f 0.1 and 0.6) are present in each of the petroleum and chloroform extracts of C. officinalis roots. Only the chloroform extract of the roots of C. lacciferus has an active compound (R_f 0.4). None of the root extracts of C. aromaticus showed activity.

Chromatographic fractionation of the active chloroform extract of C. lacciferus roots has provided 3 kauranoids and 2,6-dimethoxybenzoquinone (Bandara and Wimalasiri, 1987; Bandara et al., 1987a). Penduliflaworosin (Adesogan, 1981) and officinalin have been isolated from the chloroform and petroleum extracts, respectively, of C. officinalis roots by chromatographic means (Wimalasiri, 1987). Out of these six compounds, only 2,6-dimethoxybenzoquinone inhibits the growth of C. cladosporioides.

The antifungal activity of 2,6-dimethoxybenzoquinone against five species of fungi was tested using the TLC-bioassay technique. Inhibition areas corresponding to varying amounts of the compounds are given in Table 2. These results show that the compound is toxic to all the fungi tested and that relatively less material is required for the inhibition of three plant pathogenic fungi (B. theobromae, C. gloeosporioides and G. fujikuori) than for the two saprophytes (A. niger and C. cladosporioides).

2,6-Dimethoxybenzoquinone has also been isolated as one of the antifungal constituents from the fruit-shells of Limonia acidissima L. (Bandara et al., 1987b).

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Table 1. Antifungal acitivity of the extractives of three Croton species against Cladosporium cladosporioides

Plant	Plant part (kg)[a]	Extractive (g)[b]	Activity	
			$R_f(CHCl_3)$	Diameter of the zone of inhibition/mm
C. aromaticus	Roots (4.3)	Petroleum (110)	-	-
		Chloroform (33)	-	-
		Methanol (55)	-	-
	Leaves (1.8)	Steam distillate (1.1)	0.2	8
			0.4	22
			0.6	10
C.lacciferus	Roots (4.5)	Petroleum (67)	-	-
		Chloroform (42)	0.4	21
		Methanol (54)	-	-
	Leaves (2.85)	Steam distillate (14)	0.4	21
			0.6	9
C.officinalis	Roots (1.7)	Petroleum (16)	0.1	8
			0.6	15
		Chloroform (22)	0.1	10
			0.6	12
		Methanol (18)	-	-
		Benlate	0	38

[a] Weight of plant material in kg.

[b] Weight of the extractive in g.

Table 2. Area of fungal growth inhibition zones corresponding to varying amounts of 2,6-dimethoxybenzoquinone

Fungus	Inhibition area (cm^2) corresponding to varying amounts of 2,6-dimethoxy-benzoquinone					Amount (mg) required[a] for an inhibition area of 1.0 cm^2
	200 g	100 g	50 g	25 g	12.5 g	
Cladosporium cladosporioides	1.85	1.51	0.61	0.61	0.38	7.5×10^{-2}
Colletotrichum gloeosporioides	1.93	1.51	1.27	0.97	0.67	2.6×10^{-2}
Aspergillus niger	2.17	1.70	1.21	0.62	-	4.0×10^{-2}
Botrydiplodia theobromae	3.67	3.18	1.98	1.32	0.66	1.8×10^{-2}
Gibberella fujikuroi	3.00	2.43	1.88	1.53	0.61	1.0×10^{-2}

[a] The amount required for an inhibition area of 1.0 cm^2 was deduced from the plots, inhibition area vs. amount.