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FUNGICIDAL ACTIVITY OF SYNTHETICALLY MODIFIED CASHEW NUT SHELL LIQUID

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Abstract: Three synthetic modifications of cashew nut shell liquid were prepared with a view of increasing its mild fungicidal activity. Of these the potassium dichromate oxidized cashew nut shell liquid showed the highest activity in the tlc-*Cladosporium* bio-assay. The oxidized product inhibited the growth of several fungal plant pathogens examined. The active compound was isolated by silica gel column chromatography and identified as a biodegradable quinone produced by the aromatic ring oxidation of cardol.

INTRODUCTION

Sri Lankan farmers have traditionally used dried, powdered cashew leaves and fumigation with cashew nut shells to protect vegetable crops from insects and fungi. The pericarp of the nut contains a mixture of phenolic compounds of anacardic acid, cardol and anacardol.[1] We have previously shown that cashew nut shell liquid has a mild fungicidal activity.[2] The development of inexpensive fungicides based on locally available natural products as an alternative to imported fungicides is an attractive proposition. Synthetic modifications were prepared from cashew nut shell liquid with a view of improving its mild fungicidal activity in this investigation. The activity of the synthetic modifications were examined against several fungi that are pathogenic to plants. Active compounds were isolated and their biodegradation also investigated.

METHODS AND MATERIALS

Preparation of synthetic modifications from cashew nut shell liquid

Acetylated cashew nut shell liquid: Cashew nut shell liquid (2g) was dissolved in pyridine (5 ml), at 0° C and acetic anhydride (2 ml) was added. The mixture was left at 4° C for 18 h, diluted with methylene chloride and repeatedly washed with water. Concentration of the solvent gave the acetylated product.

Nitrated cashew nut shell liquid: Cashew nut shell liquid (2g) was dissolved in a 1:1 mixture of concentrated HNO_3 and H_2SO_4 and left at 0°C for 1.5 h, diluted with methylene chloride and washed with water. The organic layer was dried and concentrated.

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Potassium dichromate oxidation of cashew nut shell liquid: Cashew nut shell liquid (2.5 g) was dissolved in 10% aqueous sulphuric acid (100 ml), and potassium dichromate (2.5 g) was added and the mixture was stirred at 29° C for 24 h. Thereafter, the resulting solution was repeatedly extracted with methylene chloride and the combined organic layer was washed with aqueous sodium carbonate and then with water, dried and concentrated to give the oxidized cashew nut shell liquid.

Cladosporium tlc - bioassay: Test solutions were spotted on tlc plates (Merck, tlc grade silica coated on 20x20 cm glass plates) and the plates were developed in methylene chloride in solvent tanks equilibrated with the eluent. After air drying the plates at ambient temperature for 24 h, the plates were sprayed with a conidia suspension of *Cladosporium cladosporioides* and incubated as described by Smith.[3] The solvent only was used to spot the tlc plates in controls. The percentage inhibition was calculated as follows:

$$\text{Percentage Inhibition} = \frac{\text{Growth in control - Growth in test x 100}}{\text{Growth in control}}$$

Organisms: The following fungi, *Rhizoctonia solani, Poria hypolateritia, Corynespora cassiicola, Colletotrichum gloeosporioides, Sarocladium oryzae, Fusarium* sp. and *Curvularia* sp. were used as the 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 (20 mg/ml of test compound 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 7-day old fungal culture growing on PDA at room temperature, was placed at the centre of the medium. 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.*[4] The experiment was carried out in triplicate.

The percentage inhibition was calculated as follows,

$$\text{Percentage inhibition} = \frac{\text{growth area in reference-growth area in sample x 100}}{\text{growth area in reference}}$$

Isolation of active compound: Crude cashew nut shell liquid (1.2 g) was chromatographed on silica gel (35 g) eluting with methylene chloride : methanol mixtures of increasing polarity. Fractions were collected and subjected to the *Cladosporium* bioassay. Active fractions were combined and the solvent was removed *in vacuo* to obtain the pure active compound.

Degradation of the active compound: Fifteen ml of potato dextrose liquid medium was introduced into 100 ml Erlenmyer flasks and each flask was inoculated with a 0.5 cm^2 agar square of *Colletotrichum gloeosporioides* obtained from the periphery of a 7-day old culture on PDA at room temperature. To each liquid culture medium 20 mg of the test compound in 2 ml of acetone was added immediately after inoculation. The cultures were incubated at room temperature and harvested 5,7,9,11,13 days after inoculation by filtering through a Whatman no 1 filter paper. In the control experiments, the test compound was incorporated into liquid medium not inoculated with the fungus. The pH of the culture filtrate was also monitored.

The absorbance of the culture filtrates were measured at 274 nm. Biodegradation was estimated by calculating the percentage decrease in absorbance at 274 nm assuming that the degradation product has no significant absorbance. The percentage decrease in absorbance at 274 nm was calculated as follows:

$$\text{Percentage decrease in absorbance} = \frac{\text{Initial absorbance - Absorbance after harvest}}{\text{Initial absorbance}} \times 100$$

Spectroscopic Analysis : NMR was recorded in $CDCl_3$ solution, using a Bruker AM 300 spectrometer operating at 300 MHz. Mass spectrum was recorded using a Varian MAT CH 7 mass spectrometer in the EI mode. IR spectrum was recorded in $CHCl_3$ solution using a Perkin-Elmer 1420 instrument. UV absorbance was measured in 1 cm cells using a Varian DMS 90 UV-visible spectrometer.

RESULTS

Anti-fungal activity of the synthetic modifications

Of the three synthetic modifications examined by the *Cladosporium* bio-assay, only the dichromate oxidation product showed a significantly higher anti-fungal activity (Table 1). Two growth inhibitory zones, one major and one minor were clearly visible on the tlc plates.

Inhibition of the fungal growth

The potassium dichromate oxidation product inhibited the growth of the test fungi (Table 2). It was most effective against *R. solani, C. gloeosporioides, C. cassiicola* and *Curvularia* sp. The effect was low on *P. hypolateritia*. The inhibitory activity increased with the increase in concentration of the active compound and the increase was significant with *R. solani, C. gloeosporioides, S. oryzae* and *Fusarium* sp.

Table 1: Inhibition of *Cladosporium* by cashew nut shell liquid and derivatives at 0.05%.

Compound	Percentage inhibition *
Cashew nut shell liquid	6 ± 2 a
Acetylated cashew nut shell liquid	4 ± 1 a
Nitrated cashew nut shell liquid	9 ± 2 a
Potassium dichromate oxidized cashew nut shell liquid	36 ± 3 b

* Average of three replicates ± standard error of the mean.

Means in a column followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).

Isolation of the active compound

When the active fractions from the *Cladosporium* bio-assay were combined and solvent removed *in vacuo*, 0.367 g of the pure active compound was isolated after column chromatography. The active compound was identified as the quinone resulting from the oxidation of cardol.

The following spectral data were obtained for this compound,

UV (MeOH) 274 nm (=12,400), 225 nm (=4,100).
IR ($CHCl_3$) 1643, 1605 cm^{-1}.
^1H NMR ($CDCl_3$) 10.20 (1H brs, exchangeble with D_2O) 7.37. (1H,s), 6.82(1H,s), 5.10-5.45(4H,m), 2.75-3.0(8H,m), 1.2-2.0(15H,m).
MS m/e 330, 329, 207, 123.
Analysis observed: C 76.66%, H 9.24%.
Calculated for $C_{21}H_{30}O_3$: C 76.31%, H 9.16%.

Based on the above data, the following quinone structure was proposed for the active compound.

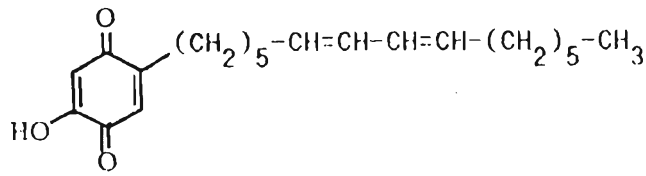

Table 2: Inhibition of fungal growth by the potassium dichromate oxidized cashew nut shell liquid.

Concentration of test compound	Percentage inhibition of growth* 0.01%	0.02%	0.05%
Fungus			
Rhizoctonia solani	57 ± 5 a	83 ± 4 b	86 ± 5 b
Poria hypolateritia	12 ± 3 c	14 ± 2 c	15 ± 3 c
Corynespora cassiicola	42 ± 3 d	46 ± 2 d	48 ± 4 d
Colletotrichum gloeosprioides	61 ± 1 e	78 ± 4 f	82 ± 2 f
Sarocladium oryzae	10 ± 2 g	27 ± 1 h	31 ± 3 h
Fusarium sp.	29 ± 3 j	45 ± 4 k	49 ± 2 k
Curvularia sp.	48 ± 5 l	57 ± 5 l	61 ± 2 l

* 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 range test).

Degradation of the active compound

The results show that the absorbance at 274nm decreased when incubated with the fungus *C. gloeosporioides* (Table 3). The decrease in absorbance at 274 nm indicate the degradation of the active compound cardol quinone. In the control experiment, without the fungus a decrease in absorbance was not observed.

Table 3: The percentage decrease in absorbance at 274nm in the culture filtrates of *Colletotrichum gloeosprioides.*

Days after inocultion	Percentage decrease in absorbance *	pH of culture test	Filtrate control
5	21 ± 1 a	6.7	7.4
7	24 ± 2 a	7.7	7.5
9	24 ± 1 a	8.2	7.5
11	27 ± 2 a	8.1	7.4
13	28 ± 2 ab	8.1	7.2
15	34 ± 1 b	8.1	7.1

* Average of three replicates ± standard error of the mean.

Means in a column followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).

DISCUSSION

Mild fungicidal activity in cashew nut shell oil can be significantly increased by oxidation with potassium dichromate. The oxidized product is effective against a wide variety of fungal pathogens. The major active compound in the preparation is a quinone resulting from the aromatic ring oxidation of the cardol. Room temperature dichromate oxidation of the cardol in the acidic medium is chemoselective as the alkene side chain and the double bond are not degraded in the reaction.

The cardol quinone undergo biodegradation when applied to the liquid culture medium. The degradation must occur in the quinone moiety of the molecule as a decrease in absorption is observed at the monitoring wavelength of 274 nm, which is the UV absorption peak of the quinone chromophore. The investigation showed that, cardol quinone has significant anti-fungal activity and is biodegradable, and that this compound therefore has the potential to be developed as an effective fungicide.

References

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