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Original Article

Additional studies on the antifungal activity of a methanol extract of *Ipomoea carnea* subsp. *fistulosa* and Octadecyl *p*-Coumarates

Eugene Sebastian John Nidiry[1*], Girija Ganeshan[2], Ankanahalli Narayanashetty Lokesha[1], Nanjundagowda Ramachandran[2]

[1]Section of Medicinal Crops, Indian Institute of Horticultural Research, Hessaraghatta Lake post, Bangalore-560089, INDIA.
[2]Division of Plant Pathology, Indian Institute of Horticultural Research, Hessaraghatta Lake post, Bangalore-560089, INDIA.

ABSTRACT

Background: *Ipomoea carnea* subsp. *fistulosa* is a plant native to South America, but sparsely distributed in India and Bangladesh. Aphrodisiac, purgative, cathartic and sore curing properties have been attributed to the plant. Although there were several papers on the antifungal and antibacterial activities of on the crude extractives of the plant, the first report on the bioassay monitored isolation and characterization of the chief antifungal compounds as a mixture of E and Z isomers of octadecyl p-coumarates was published from this Institute. **Material and Methods:** The methanol extractive of *Ipomoea carnea* subsp. *fistulosa* exhibited antifungal activity against the mycelial growth of *Phytophthora nictotiana* by poisoned food technique. This is in addition to the reported activity against *Colletotrichum gloeosporioides*. **Results and Discussion:** Octadecyl p-coumarates isolated from the plant by column chromatography followed by HPLC exhibited activity against the mycelial growth of *Cercospora capsici*. This is in addition to the reported activity of octadecyl p-coumarates against the spore germination of *Alternaria alternata*, *Alternaria porri* and *Cladosporium cucumerinum*. **Conclusion:** Additional studies conducted with the crude extractive of *Ipomoea carnea* subsp. *fistulosa* confirmed its antifungal activity against *Phytophthora nicotianae*. Identification of octadecyl p-coumarates as antifungal active principles was confirmed by its activity against *Cercospora capsici* by modified mycelial growth inhibition study.

Key words: *Ipomoea carnea* subsp. *fistulosa*, Octadecyl*p*-coumarates, Antifungal activity, *Phytophthora nicotianae*, *Cercospora capsici*.

Correspondence:
Dr. Eugene Sebastian J Nidiry, Section of Medicinal Crops, Principal Scientist, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore-560 089, INDIA.

E-mail: nidiry@yahoo.co.in
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INTRODUCTION

Ipomoea carnea subsp. *fistulosa* is a plant native to South America, but sparsely distributed in India and Bangladesh.[1] Aphrodisiac, purgative and cathartic properties have been attributed to the plant.[2] The leaf paste of the plant is applied on sore between toes and fingers.[3]

Isolation and chemical characterization of resinous glycosides,[4] flavonolglycosides[5-6] and alkaloids[7] from the leaves and anthocyanin from the flowers[8] of *I. carnea* have been reported. The leaves are toxic to cattle and the toxicity is attributed to the inhibitory activity of lysosomal β-glucosidase and α and β-mannosidases by polyhydroxy alkaloids such as swainsonine and calystegines.[9]

Although there were severalpapers on the antifungal and antibacterial activities of on the crude extractives of the plant, the first report on the bioassay monitored isolation and characterization of the chief antifungal compounds as a mixture of *E* and *Z* isomers of octadecyl p-coumarates (Figure 1) was published from this Institute.[10]

Detailed spectral studies of octadecyl p-coumarates and tentative detection of other alkyl coumarates by High Resolution Electro Spray Ionization Mass Spectrometry (HRESIMS) were also subsequently reported.[11-13]

This paper deals with the additional studies on antifungal activity of the methanol extract and octadecyl *p*-coumarates isolated from the plant. Thus the crude methanol extract was tested against the mycelial growth of *Phytophthora nicotianae,* and *Fusarium oxysporum. P. nicotianae* is the causal organism of phytophthora neck and bulb rot of onion. *F. Oxysporum* is the causal organism vascular wilt, yellows, corm rot, root rot and damping-off in potato, cowpea other horticultural crops. Octadecyl p-coumarates isolated from the plant was tested against the mycelial growth of *Cercospora capsici* which causes a foliar disease of chilli known as leaf spot.

MATERIALS AND METHODS

Poisoned food technique

Preparation of the extract

A mass of 100 g. of fresh leaves of *Ipomoea carnea* subsp. *fistulosa* were collected and dried at 60°C and were powdered. The dry powder (25 g.) was exhaustively extracted with methanol using a Soxhlet apparatus (10 extractions). The methanol was completely distilled out to get the extract (4.5 g).

Mycelial growth inhibition of *Phytophthora nicotianae* and *Fusarium oxysporum* was evaluated by the Poisoned food technique.[14] Methanol extract was added to the sterilized media of potato–dextrose-agar (PDA) to get a concentration of 5000 mg/L. Surfactant Tween 20 was added at 0.3% level to the media before plating to get uniform emulsion of the extract, the same amount of surfactant being added to the control also. Mycelia discs of 10 day old cultures of *P. nicotianae* and *F. oxysporum* were placed at the centres of the Petri plates and mycelial growth was measured after an incubation period of 6 days. The results are presented in Table 1.

Table 1: Fungitoxicity of crude methanol extractive (5000 mg/L) of *Ipomoea carnea* subsp. *fistulosa*

Test organism	Per cent mycelial growth inhibition w.r.t control
Phytophthora nicotianae	29.8
Fusarium oxysporum	0.0

Table 2: Growth inhibition of *Cercospora capsici* on initial treatment of mycelial discs with octadecyl *p*-coumarates

Treatments	Observation on mycelial growth of *Cercospora capsici* in cm					
	2nd Day		3rd Day		4th Day	
	Diam (cm)	Per cent inhibition w.r.t. control*	Diam (cm)	Per cent inhibition w.r.t. control	Diam (cm)	Per cent inhibition w.r.t. control
Sterilized water	3.11	-	4.78	-	6.13	-
10% Acetone in water (Control)	3.09	-	4.64	-	5.74	-
Octadecyl p-coumarates (250 mg/L)	2.98	4.80	4.60	1.04	5.70	0.81
Octadecyl p-coumarates (500 mg/L)	1.98	51.5	3.86	20.31	5.09	23.3

*Percent mycelial growth inhibition was calculated after giving due adjustment of initial diameter of the mycelial discs (0.8 mm).

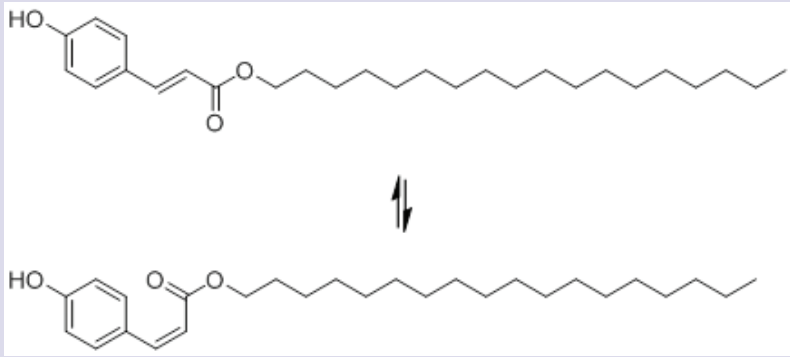

Figure 1: *E* and *Z* isomers of octadecyl p-coumarates.

Isolation and characterization of octadecyl p-coumarates

Isolation and purification were done by column chromatography followed by 5 stages of HPLC purification. Chemical characterization was done by spectral studies involving UV, IR, CIMS, HRESIMS, H^1 NMR. C^{13} NMR and 2D NMR (H-H COSY and HSQC). Details are given in our earlier papers.[10]

Modified mycelial growth inhibition studies using treated mycelial discs

The poisoned food technique[14] required substantial amounts of material and thus this study could not be carried out for the pure octadecyl p-coumarates because of the paucity of the material which was obtained in milligram amount only after five stages of HPLC purification. Thus modifying the growth inhibitory assay based on the method of Corden and Young[15] (1962) was adopted.

Mycelial discs (8 mm diameter) of 14 day old cultures *Cercospora capsici* were taken using sterilized cork borer and were dipped in 250 ppm and 500 ppm solutions of octadecyl *p*-coumarates in 10% acetone in water for 1 h and inoculated at the centre of the Petri plate. Mycelial growth was recorded by measuring the diameter in cm every day. For control, a mycelial disc dipped in 10% acetone in water was used. Table 2 gives the observations obtained for *C. capsici.*

RESULTS AND DISCUSSION

The results of the bioassay of the crude methanol extractive at a concentration of 5000 mg/l on the mycelial growth of *Phytophthora nicotianae*, and *Fusarium oxysporum* by poisoned food technique are presented in Table 1. The results show that the crude extractive exhibits mycelial growth inhibition of *P. Nicotianea* but not that of *F. oxysporum*. It may be noted that in our earlier paper[10] the activity of the crude extractive against the mycelial growth of *Colletotrichum gloeosporioides* was already reported. Thus the present report on the activity of the extractive against *P. nicotianae* is in addition to the earlier report on the activity against *C. gloeosporioides.*

Bioassay monitored isolation using *C. gloeosporioides* and *C. cucumerinum* as test organisms led to the isolation and identification*E* and *Z* isomers of octadecyl *p*-coumarates as whose 3D Structures are given below (Figure 2). In these structures, green colour depicts carbon atoms, blue colour depicts hydrogen atoms and red colour depicts oxygen atoms.

However, after five stages of HPLC purification, only 30 mg of octadecyl *p*-coumarates could be obtained from 2 Kg of dried plant material. In our earlier paper, we had confirmed the antifungal activity of the compounds against the spore germination of *Alternaria alternata* and *A. porri.* However, the activity of pure octadecyl *p*-coumarates against the mycelial growth could not be confirmed because substantial amount was required for the poisoned food technique.

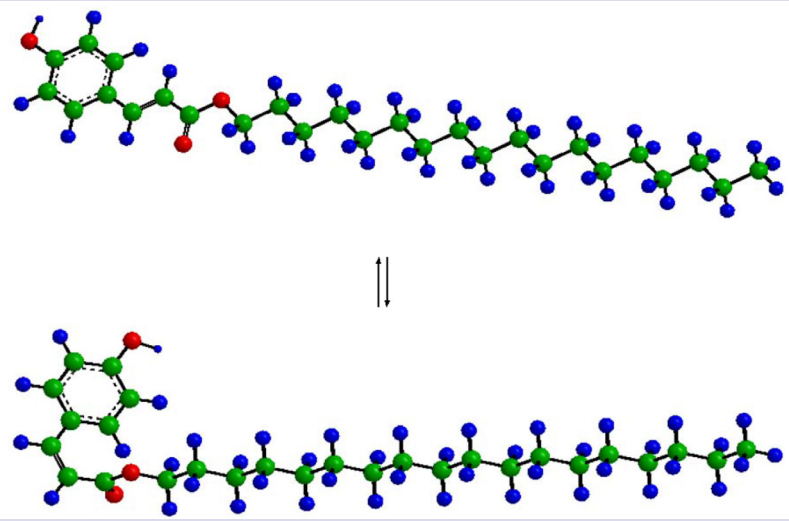

Figure 2: 3D Structures of *E* and *Z* isomers of octadecyl *p*-coumarates.

In view of this problem, we adopted the modified mycelial growth inhibition study following Corden and Young method using *Cercospora capsici* as test organism. The results are presented in Table 2.

The results clearly show that octadecyl *p*-coumarates at concentration of 500 mg/L inhibits the mycelial growth of *C. capsici*.

CONCLUSION

Additional studies conducted with the crude extractive of *Ipomoea carnea* subsp. *fistulosa* confirmed its antifungal activity against *Phytophthora nicotianae*. Identification of octadecyl p-coumarates as antifungal active principles was confirmed by its activity against *Cercospora capsici* by modified mycelial growth inhibition study.

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ABBREVIATION USED

HRESIMS: High Resolution Electro Spray Ionization Mass Spectrometry; **HPLC:** High Performance Liquid Chromatography; **UV:** Ultra Violet; **IR:** Infra Red; **CIMS:** Chemical Ionization Mass Spectrometry; **NMR:** Nuclear magnetic resonance; **COSY:** Correlation Spectroscopy; **HSQC:** Heteronuclear Single-Quantum Correlation Spectroscopy.

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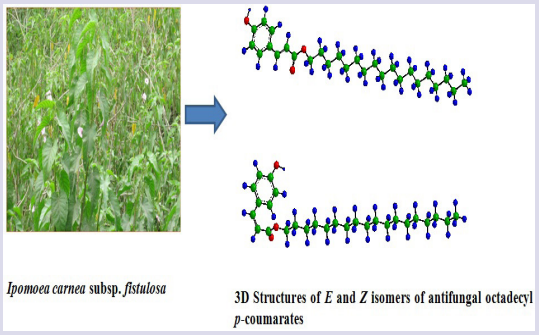
SUMMARY

- The crude extractive at a concentration of 5000 mg/l exhibits mycelial growth inhibition of *Phytophthora nicotianae*, but not that of *Fusarium oxysporum*.
- Bioassay monitored isolation using *C. gloeosporioides* and *Cladosporium cucumerinum* as test organisms had led to the isolation of E and Z isomers of octadecyl p-coumarates.
- Confirmed the antifungal activity of the octadecyl p-coumarates against the spore germination of *Alternaria alternate* and *A. porri*.
- The results from the modified mycelial growth inhibition study clearly showed that octadecyl p-coumarate inhibits the mycelial growth of *Cercospora capsici*.

PICTORIAL ABSTRACT



ABOUT AUTHORS

Dr. Eugene Sebastian J Nidiry: Is Principal Scientist (Organic Chemistry) in the Section of Medicinal Crops of Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore. His main interest is in the structure elucidation of biologically active natural compounds and structure-activity relationship studies.

Dr (Mrs). Girija Ganeshan: Is Principal Scientist (Plant Pathology) in the Division of Plant Pathology of Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore. Her main interest is in the control of fungal diseases of vegetable crops using natural and synthetic compounds.

Mr. A.N. Lokesha: Is Senior Technical Assistant in the Section of Medicinal Crops of Indian Institute of Horticultural Research, Hessaraghatta Lake post, Bangalore. His main interest is in the method development and analysis of active ingredients in different medicinal Crops using chromatographic techniques.