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Studies on essential oils, Part 33: chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* Lour.

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ABSTRACT: Chemical investigations of the leaf essential oil of *Coleus amboinicus* by GC and GC–MS techniques indicated the presence of six components, accounting for 97% of the total oil. The major component was thymol (94.3%), followed by carvacrol (1.2%), 1,8-cineole (0.8%), *p*-cymene (0.3%), spathulenol (0.2%), terpinen-4-ol (0.2%) and an unidentified component (1.4%). The oil was insecticidal to white termites (*Odontotermes obesus* Rhamb.) with 100% mortality at a dose of 2.5×10^{-2} mg/cm^3 for 5 h exposure. This oil was also more active than the synthetic insecticides, Thiodan and Primoban-20, against termites, although it was ineffective against *Tribolium castaneum,* a stored product pest. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: *Coleus amboinicus*; chemical composition; insecticides; *Odontotermes obesus*; essential oil

Introduction

Coleus amboinicus Lour. (syn. *Coleus aromaticus* Benth.; Labiatae) is an evergreen aromatic herb growing in gardens throughout India and is native to East India. It is commonly known as 'country borage' or 'Indian borage' or in Hindi as 'pathorchur'. The leaves have a pleasant aromatic odour, a pungent taste and are used for flavouring meats, salads and also a substitute for borage (*Borago officinalis* Linn.), which is used for flavouring wines and beer. The leaves are reported[1] to be useful for urinary diseases, chronic coughs, epilepsy, fever, conjunctivitis, dyspepsia, asthma and colic disorders. Several natural products have been previously isolated from the plant[2–9] but studies on the chemical composition of the volatile oil from the foliage of this plant are quite meagre.[10,11] The antifungal and antibacterial activities of the leaf oil have also been studied against pathogenic bacteria and fungi.[12] In continuation of our research programme[13–18] on essential oils, chemical and insecticidal investigations of the volatile oil obtained by hydrodistillation were undertaken and the results are reported in this communication.

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Experimental

Plant Material and Isolation of Oil

Leaves of *Coleus amboinicus* (1 kg) were collected in the month of September from Rajahmundry, Andhra Pradesh, and the voucher specimen of the plant is kept in the herbarium of the Science Faculty, D.D.U. Gorakhpur University, Gorakhpur. The leaves were cut into small pieces, washed with distilled water and the volatile oil obtained by hydrodistillation (5 h) using Clevenger's apparatus. The light-yellow oil (yield 0.6%) thus obtained was dried over anhydrous sodium sulphate and stored at 4 °C.

Gas Chromatography

The GC chromatogram of the oil was obtained using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and two HP fused silica columns (A and B). Column A was an HP-5 (length 30 m × 0.32 mm) whose injector and detector (FID) temperatures were maintained at 250 °C and 270 °C, respectively. The amount of sample injected was 0.1 μl (in split mode) and the flow rate was fixed as 1.2 ml/min. The oven temperature was programmed as follows: 60 °C for 5 min, rising at 1 °C/min to 140 °C, then at 10 °C/min to 270 °C and held for 5 min. Column B was an HP-Innowax (length 30 m × 0.53 mm) with injector and detector temperatures at 250 °C. The same amount of sample was injected in split mode with a flow rate as 2.5 ml/min. Nitrogen was used as the carrier gas and the oven temperature was programmed as follows: 60 °C for 5 min, rising at 1 °C/min to 140 °C, then at 10 °C/min to 240 °C and held for 5 min.

GC–MS

The GC–MS analysis of the oil was undertaken using a Hewlett Packard HP 6890 series GC fitted with a Hewlett Packard Mass Detector (Model 5973) and a HP-5 column 30 m × 0.25 mm i.d. (crosslinked 5% phenyl methyl siloxane). The injector, GC–MS interphase, ion source and selected mass detector temperatures were maintained at 270 °C, 280 °C, 230 °C and 150 °C, respectively. Helium was used as the carrier gas and the oven temperature was programmed as follows: 60 °C for 5 min, rising at 1 °C/min to 140 °C, then at 10 °C/min to 270 °C and held for 5 min.

Identification of Components

Chemical constituents were identified by comparing their mass spectra with the library NBS 75 K and/or by co-injection with authentic samples, and the results are reported in Table 1.

Insecticidal Activity

The insecticidal activity of the oil against white termites (*Odontotermes obesus* Rhamb.) was determined using a glass petri dish (80 mm diameter). The required quantity of oil (0.6×10^{-2}, 2.5×10^{-2}, 3.9×10^{-2} and 7.7×10^{-2} mg oil/cm^3) was applied to a piece of filter paper (10 mm diameter), pasted on the inner surface of the cover of the petri dish. A group of 10 termites, along with 10 g soil and small pieces of sugarcane stem, were placed inside each petri dish in both the treated and the control sets. There were three replicates for each treatment group.

Sublethal toxicity of the oil was also investigated by observing the recovery of immobilized termites after transferring them to a fresh petri dish. The efficacy of this oil was also compared with two commercial synthetic insecticides, endosulphan 35% (Thiodan) and chlorpyriphos 20% (Primoban), and the results are reported in Table 2.

The insecticidal activity of the oil against the confused flour beetle, *Tribolium castaneum*, was also investigated

Table 1. Chemical composition of *C. amboinicus leaf* oil

Component	Retention Indices*	%
p-Cymene	1028	0.3
1,8-Cineole	1035	0.8
Terpinen-4-ol	1178	0.2
Thymol	1291	94.3
Carvacrol	1300	1.2
Spathulenol	1576	0.2
Unidentified component	1940	1.4
Other minor components	—	1.6

* In HP-5 capillary column.

Table 2. Insecticidal activity of *C. amboinicus* leaf oil and synthetic insecticides against white termites (*O. obesus*)

Volatile oil/synthetic insecticides	Dose (mg/cm^3)	Mortality* at different exposure duration(%)						
		1h	2h	3h	5h	7h	12h	24h
C. amboinicus	0.6×10^{-2}	20	30	40	60	60	70	80
	2.5×10^{-2}	30	70	90	100	100	100	100
	3.9×10^{-2}	30	80	90	100	100	100	100
	7.7×10^{-2}	80	100	100	100	100	100	100
Chlorpyriphos, 20% (Primoban-20)	0.6×10^{-2}	0	0	0	30	80	90	100
	2.5×10^{-2}	0	10	50	80	100	100	100
	3.9×10^{-2}	0	20	70	100	100	100	100
	7.7×10^{-2}	0	30	100	100	100	100	100
Endosulfan, 35% (Thiodan)	0.6×10^{-2}	0	0	10	20	60	80	90
	2.5×10^{-2}	0	10	20	30	80	100	100
	3.9×10^{-2}	0	10	20	40	80	100	100
	7.7×10^{-2}	0	30	40	70	100	100	100
Control	0	0	0	0	10	20	20	30

* Average of three replications.

in a similar manner but with wheat seeds in the petri dishes instead of soil and sugarcane stems.

Results and Discussion

Analysis of the leaf oil by GC and GC–MS indicated the presence of six components, accounting for 97% of the total weight of the oil. The major component was thymol (94.3%), followed by carvacrol, 1,8-cineole, *p*-cymene, spathulenol, terpinen-4-ol and an unidentified component. The unidentified component has MS (*m/z*, rel. int.): 150 (52), 135 (100), 109 (21), 91 (12), 77 (4),67 (3), 55 (5), 43 (11). Baslas *et al.*[10] reported that the oil from this species contained thymol (41.3%), carvacrol (13.3%), 1,8-cineole (5.5%), eugenol (4.4%), β-caryophyllene (4.2%), terpinolene (3.8%), α-pinene (3.2%), β-pinene (2.5%) and β-phellandrene (1.9%) as the major constituents. Recently, Pino *et al.*[11] reported that the leaf oil of this plant from Cuba contains 20 components, with carvacrol (64%) as the major constituent. In another study,[19] carvacrol was also identified as the major constituent, followed by thymol, eugenol, chavicol, ethyl salicylate, methyl eugenol, methyl chavicol, caryophyllene, 1,8-cineole, α-terpineol and *p*-cymene. In yet another study, Wechuizen[8,9] indicated that the leaf oil of this species contained 50% carvacrol. Clearly there are major differences in chemical composition between samples of this species that may result from genetic, environmental, developmental or other differences.

Bioassay (Table 2) of this volatile oil against white termites (*Odontotermes obesus* Rhamb.) showed that efficacy is a function of both dose of oil and duration of exposure: 2.5×10^{-2} mg oil/cm^3 caused complete mortality within 5 h of exposure; however, 7.7×10^{-2} mg/cm^3 gave complete mortality at 2 h. Termites that were immobilized did not recover when moved to an untreated petri dish. In comparison to tested synthetic insecticides, the oil was more efficacious (Table 2). However, the oil was ineffective against *Tribolium castaneum*. The major component of the oil, thymol, is reported to have antibacterial,[20] antifungal,[21] insecticidal and genotoxic,[22,23] activities. This indicates that the insecticidal activity of the oil may be due to this major component.

Summarizing these results, it may be concluded that the leaf oil of *Coleus amboinicus* from India is rich in thymol and is a potent insecticide against *O. obesus,* a pest of sugarcane fields.

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