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Chemical and biocidal investigations on rhizome volatile oil of *Curcuma zedoaria* Rosc—Part 32

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Chemical investigations on *Curcuma zedoaria* rhizome volatile oil, using HPLC, GC and GC-MS techniques, showed the presence of 1,8-cineole (18.5%), *o*- and *p*-cymene (18.42%) and α-phellandrene (14.93%) as major constituents followed by terpinolene (4.11%), α-pinene (3.28%), β-turmerome (3.1%), β-pinene (2.93%), β-phellandrene (2.0%), etc. This oil was found to be highly insecticidal against *Odontotermes obesus* Rhamb. (white termite) and the minimum dose for 100% mortality was recorded as 2μL per petriplate for 24 h exposure duration. It also showed complete mycelial inhibition of *Colletorichum falcatum* at 4 μL per petriplate of the oil and was ineffective in controlling the mycelial growth of *Aspergillus terreus, Fusarium graminearum, F. solani* and *Curvularia pallescens.*

Curcuma zedoaria Rosc. is commonly known as 'Zedoary' and simply 'Kachura' and distributed in many parts of India. It is native of north east India, growing in eastern Himalayas and closely resembles to *Curcuma longa* in appearance. A highly valued commercial product "Shoti Starch" (82.6% starch) is extracted from rhizomes of the plant. The rhizomes are also used[1,2] for jaundice, as blood purifier, for promoting vital energy circulation, removing blood stasis, promoting digestion, in alleviating pain and in preparation of useful products such as 'Abir' (a red powder), perfumery and cosmetics.

Rhizomes on steam distillation or solvent extraction give light yellow oil. A number of natural products were extracted[2-12] from rhizomes of this plant. This oil has good medicinal value, it possess inhibitory effect on platelet activating factor for rabbit platelets[13], low pupation, adult emergence, insecticidal and antifeedant activities against *Pluetella xylostella*[14] and is also toxic to neonate larvae of *Spodoptera lettoralis*[9]. The sesquiterpenes, curzerenone and curcumenol, isolated from the rhizomes, possess pharmacological activity[11] in mice and rats.

The rhizome volatile oil extracted by various solvents has been well-investigated[2-12] but oil obtained by hydrodistillation is not yet been investigated. In continuation of our research programmes[15-20], the chemical, antifungal and insecticidal studies on the rhizome volatile oil of this plant have been undertaken and the results are reported in this communication.

Experimental Procedure

Isolation of volatile oil

The rhizomes of *Curcuma zedoaria*, collected from Rajamundry, Andhra Pradesh, were cut into small pieces, washed with distilled water and the volatile oil was obtained by hydrodistillation using Clevenger's apparatus. The light yellow oils thus obtained was dried over anhydrous sodium sulphate and stored under refrigeration.

Chemical analysis of volatile oil

The chemical composition of rhizome volatile oil was investigated by HPLC, GC and GC-MS techniques.

HPLC analysis

The essential oil was subjected to HPLC analysis using DATALAB 3103 UV-VIS detector coupled with a 3101 pump and an Alltech Econosil C-18 5U column (250 mm×4.6mm). The 25 μL sample (without dilution) was injected using methanol and water (9:1 ratio) as mobile phase. The run rate was

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fixed at 1 mL per min and chromatogram was recorded at 254 nm. The HPLC chromatogram showed 12 peaks.

GC and GC-MS analysis

The GC and GC-MS analysis of the oil was undertaken using HP 6890 series GC fitted with a HP-5 column having 30 m length and 0.25 mm diameter (cross-linked 5% phenyl methyl siloxane). The GC apparatus was coupled with a FID detector and oven temperature was programmed from 65-75°C (1°C min^{-1}), 75-81°C (0.5°C min^{-1}) and 81-185°C (4°C min^{-1}).

Identification of components

The chemical constituents were identified by comparing their mass spectra (library spectra) and co-injection with authentic samples available. Results are included in Table 1.

Insecticidal activity

The insecticidal activity of the oil against white termite (*Odontotermes obesus* Rhamb) from sugarcane fields was investigated using 80 mm glass petriplate. The required quantities (1, 2, 3 and 6 µL per petriplate) of oil were soaked in a piece of filter paper (10 mm dia) and pasted on the inner surface of the cover of the petriplates. A group of ten termites alongwith 10 g soil and small pieces of sugarcane stem were placed inside each petriplate in both treated and control sets.

The cidal nature of the oil was also investigated by observing the revival of life of tested termite after transferring them to a fresh petriplate. The insecticidal efficacy of the oil was compared with two commercial synthetic insecticides namely endosulphan 35% (Thiodan) and chlorpyriphos 20% (Primoban-20) and the results are reported in Table 2.

Antifungal activity

The antifungal activity of the oil was investigated against *Curvularia pallescens* (CP), *Colletotrichum falcatum* (CF), *Aspergillus niger* (AN), *Fusarium solani* (FS), *Fusarium graminearum* (FG) and *Aspergillus terrus* (AT) using the inverted petriplate method[15]. The fungal cultures were maintained in an oatmeal agar medium. For antifungal studies, required doses of the oil (2, 4 and 6 µL per petriplate) were soaked in a presterlised filter paper discs (15 mm dia) pasted on the inner surface of cover petriplate and placed in an inverted position. Each test was repeated three times and fungitoxicity was recorded in terms of percent mycelial inhibition (Table 3) which was calculated by using the formula as:

$$\% \text{ mycelial inhibition} = D_c - D_t \times 100 / D_c$$

where, D_c and D_t are average diameters of mycelial colony in control and treated sets respectively.

Table 1—Chemical composition of *C. zedoaria* rhizome volatile oil

Components	RT[a]	Percentage[b]
α-Thuzene	14.99	0.15
α -Pinene	15.7	3.28
Camphene	18.59	tr
Sabinene	19.57	0.36
β-Pinene	20.03	2.93
6-Methyl-5-hepten-2-one	20.56	0.15
Myrcene	21.35	1.62
Octanal	22.69	0.24
α -Phellandrene	23.29	14.93
δ-3-Carene	23.93	1.18
α-Terpinene	24.72	0.25
o- and *p*-Cymene	25.99	18.42
β-Phellandrene	26.50	2.00
1,8-Cineole	26.94	18.50
(z)- β-Ocimene	27.57	0.40
(E)- β-Ocimene	28.94	0.20
γ-Terpinene	30.22	0.74
cis-Sabanene hydrate	31.05	0.06
cis-Linalool oxide	31.58	0.06
Terpinolene	33.17	4.11
Linalool	34.01	1.80
Citronellol	37.79	0.04
Terpinen-4-ol	39.54	0.68
p-Cymene-8-ol	39.98	1.84
Nerol	42.86	0.20
β-Caryophyllene	51.15	0.91
β-Turmerone isomers	-	3.10
ar-Turmerone	-	1.60
Various minor unidentified components	-	20.27

[a]Retention time in min. from capillary GC.

[b]Percentage were taken from the capillary GC traces with FID when available or directly from the GC-MS percentage of total ion current of the peak.

tr-Traces

Results and Discussion

HPLC, GC and GC-MS analysis led to the identification of more than 28 components listed in Table 1. The major components were 1,8 cineole (18.5%), *o*- and *p*-cymene (18.42%), α-phellandrene (14.93%) followed by terpinolene (4.11%), α-pinene (3.28%), β-turmerome (3.1%), β-pinene (2.93%), β-phellandrene (2.0%), *p*-cymene-8-ol (1.84%), linalool (1.8%) and myrcene (1.62%). The presence

Table 2—Insecticidal activity against *Odontotermes obesus* Rhamb compared with synthetic insecticides

Volatile oil/Synthetic insecticides	Dose* (in μL)	% Mortality** at different exposure duration						
		1	2	3	5	7	12	24
Curcuma zedoaria (rhizome oil)	1	0	10	20	40	50	80	80
	2	10	20	20	40	60	80	100
	3	30	60	70	90	100	100	100
	6	30	70	80	100	100	100	100
Endosulfan-35% (thiodan)	1	0	0	10	20	70	90	100
	2	0	10	20	30	80	100	100
	3	0	10	20	40	80	100	100
	6	0	20	40	70	100	100	100
Chloropriphos-20% (Primoban-20)	1	0	0	10	40	90	100	100
	2	0	10	50	80	100	100	100
	3	0	10	70	90	100	100	100
	6	0	30	100	100	100	100	100
Control	0	0	0	0	0	0	0	0

*Dose of oil in μL per 80 mm petriplate
**Average of three replications

Table 3—Antifungal activity of *Curcuma zedoaria* rhizome volatile oil

S. No	Fungus	% mycelial zone inhibition ** at different dose*of oil (μL)		
		2	4	6
1	*Curvularia pallescens*	50	60	80
2	*Colletotrichum falcatum*	79	100	100
3	*Aspergillus niger*	0	0	7
4	*Fusarium solani*	10	15	26
5	*Fusarium graminearum*	13	20	34
6	*Aspergillus terrus*	57	69	75

*Dose of oil in μL per petriplate
**Average of three replications

of various natural products *viz* sesquiterpenes and their alcohol derivatives have been reported by earlier workers[2-12].

The insecticidal studies (Table 2) of this volatile oil against *Odontotermes obesus* Rhamb showed that the dose of the oil and percent mortality depended on exposure duration. At 2 μL per petriplate dose, the 100% mortality was observed within 24 h exposure duration. However, 6 μL per petriplate dose of oil was responsible for 100% mortality within 5 h exposure duration only. A partial mortality was observed at other tested doses and exposure duration. The mortality of termites was found to be cidal in nature and minimum dose for 100% mortality was recorded as 2 μL per petriplate for 24 h exposure. A comparative insecticidal activity of oil with synthetic insecticides showed that it was less active than synthetic insecticides (Thiodan and Primoban-20).

The 100% mycelial inhibition of CF at 4 μL per petriplate dose of oil was also observed (Table 3). It was also effective in controlling the mycelial growth of AT, FG, CP and FS but not effective against AN.

It could be concluded that the rhizome volatile oil of *C. zedoaria* was rich in 1,8-cineol, *p*-cymene, α-phellandrene and has a potent antifungal as well as insecticidal activity.

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