Research Article

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VOLATILE OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *CURCUMA OLIGANTHA* VAR. *LUTEA* RHIZOMES

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ABSTRACT

Hydrodistilled volatile oil obtained from the rhizomes of *Curcuma oligantha* Trimen var. *lutea* (Zingiberaceae) was analyzed by GC and GC-MS. The volatile oil was composed mainly of cinnamyl cinnamate (48.9%), n-hexanal (14.0%) and n-octadecane (10.1%). Among the ten monoterpenes (7.1%) present in the oil, the prominent one was β-pinene (2.8%) followed by α-pinene (1.6%) and terpinen-4-ol (1.6%). The oil contained sixteen sesquiterpenes (9.7%) comprising mainly ar-turmerone (3.0%), cadinol (1.3%), eudesmol (1.2%) and bisabolol oxide (1.1%). The predominant constituents were the aromatic esters (54.4%) including cinnamyl cinnamate (48.9%) and benzyl benzoate (2.9%). The important aliphatic constituents were characterized as n-hexanal (14.0%), n-octadecane (10.1%) and isoamyl pyruvate (1.9%). About 25 components occurred in trace amounts in the oil. The volatile oil and ethanolic extract of the rhizomes showed significant antimicrobial activity.

KEY WORDS: *Curcuma oligantha* var. *lutea*, Rhizomes, Volatile oil composition, Antimicrobial activity.

INTRODUCTION

Curcuma oligantha Trimen var. *lutea*, syn. *C. cannanorensis* var. *lutea* (Zingiberaceae), is an erect perennial herb with yellow flowers. It is distributed in Sri Lanka and southern India in Karnataka and Kerala[1-4]. The Curcuma rhizomes are large, tuberous, orange-red and aromatic, substituted for turmeric and prescribed to cure skin diseases[5]. The rhizomes contained aliphatic constituents, β-sitosterol, curcumin and 6-methyl-10-cyclohex-11-enyl-n-dec-4α, 5α, 6α-triol[6]. The present paper describes chemical composition of the volatile oil and antimicrobial activity of the ethanolic extract and volatile oil isolated from the rhizomes.

MATERIAL AND METHODS

Plant Material

The rhizomes of *Curcuma oligantha* var. *lutea* were collected from Udupi, Karnataka and identified by Prof. K. G. Bhat, Taxonomist, Department of Botany, Poornaprajna College, Udupi, Karnataka. A voucher specimen No. PRL/JH/08/43 is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India.

Preparation of ethanolic extract

Air-dried rhizomes of *C. oligantha* var. *lutea* (50 g) was coarsely powdered, defatted with petroleum ether (60-80ºC) and then extracted with ethyl alcohol (95%) for 48 hours in a Soxhlet apparatus. The extract on removal of the solvent yielded a dark reddish brown viscous mass (3.1 g).

Extraction of volatile oil

The fresh rhizomes of *C. oligantha* var. *lutea* (1 kg) were hydrodistilled by using Clavenger apparatus. A pale greenish yellow essential oil (3.2 %) was obtained. It was dried over anhydrous sodium sulfate and stored at 4°C in the dark.

GC Analysis

The gas chromatographic analysis of the volatile oil was carried out on Shimadzu 2010 Gas Chromatograph (Japan) equipped with a flame ionization detector (FID) and ULBON HR-1 fused silica capillary column (60 m x 0.25 mm x 0.25 µm). The injector and detector (FID) temperatures were maintained at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 mL/min with column pressure of 155.1 kPa. The sample (0.2µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230 °C at a rate of 3° C/min and then held at 230° C for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC-MS Analysis

The GC–MS analysis of the volatile constituents was performed on a silicon DB-1 fused silica column directly coupled to the MS. The carrier gas was helium with a flow rate of 1.21 mL/min. Oven temperature was programmed as 50˚C for 1 min and subsequently held isothermal for 2 min., injector port: 250˚C, detector: 280˚C, split ratio 1:50, volume injected: 1µL of the oil. The recording was performed at 70eV, scan time 1.5 s; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station.

Identification of Compounds

The individual compounds were identified by comparing their retention indices (RI) of the peaks on ULBON HR-1 fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer

database of NBS 54 K.L, WILEY8 libraries and published literature[7-12]. Relative amounts of identical components were based on peak areas obtained without FID response factor correction. The components of the oil, the percentage of each constituent and their RI values are summarized in Table 1. The constituents are arranged in order of GLC and GC-MS elution on silicon DB-1 and ULBON HR-1 fused silica column, respectively.

Antimicrobial activity

Test Organisms and Inoculums

Pure cultures of *Escherichia coli* (NCTC-6571) and *Staphylococcus aureus* (NCTC-10418) were obtained from the Biotechnology Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Standard

Aimikacin discs with specific activity of 30 µg were obtained from the Department of Microbiology, Majeedia Hospital, New Delhi.

Media

Dehydrated nutrient agar media was prepared in distilled deionized water. The media (gm/100 ml) was composed of peptone (5.1 g), sodium chloride (5.0 g), beef extract (1.5 g), yeast extract (1.5 g) and agar (1.5 g).

Preparation of Media

Dehydrated nutrient agar medium (28 g) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

Sterilization of Media

The conical flask containing the nutrient agar medium was plugged with the help of a no*n*-absorbent cotton plug. The mouth of the conical flask and the cotton bung were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15-lbs/in^2 pressure for 20 minutes.

Preparation of Test Organisms

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37^{0}C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hours at 37+2^{0}C. The growth from the nutrient surface was washed using 50 ml of distilled water. A dilution factor was determined which gave 25 % light transmission at 530 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Temperature Control

Thermostatic control was required in several stages of a microbial assay when culturing a micro-organism and preparing its inoculums and during inoculation in a plate assay.

Cup-plate method

A previously liquefied and sterilized medium was poured in to plastic Petri-plates of 100 mm size. Sixteen plates in duplicate were prepared and kept for solidifying. Four holes were made in each plate with a stainless steel borer having 6 mm internal diameter. Different dilutions of the alcoholic extract and volatile oils of *C. oligantha* var. *lutea*, were made having concentration of 200 µg, 100 µg and 50 µg / 0.1 ml of solution. Aimikacin disc of 30 µg concentration was used as standard (S). The plates were labelled as Co (control), S (standard), A (200 µg / 0.1 ml), B (100 µg / 0.1 ml) and C (50 µg / 0.1 ml) corresponding to different holes. The plates were divided into four groups (gr-I, gr-II, gr-III & gr-IV) comprising four plates in each group. In gr-I, *C. oligantha* var. *lutea* extract was used as test solution. Each group contained 2 plates each for *E. coli* and *S. aureus*. The test solutions were made in DMSO (dimethyl sulphoxide) solvent which was used as control. Micropipette was used to deliver the solutions into the holes. The volume of solution added to each hole was kept uniform (0.1 ml in each hole). One strip of Aimikacin (standard) was placed aseptically to the centre of each plate. One hole was kept for blank (Co). The plates were then left for standing for 1 hour for proper diffusion of the drug solutions. They were incubated for about 24 hours at 32 ± 2^{o}C. After 24 hours the plates were examined and the diameters of zones of inhibition were accurately measured.

Table 1: Chemical composition of the volatile oil of *C. oligantha* var. *lutea* rhizomes

S. No.	Components	RI	%
1.	n-Hexanal	798	14.0
2.	n-Nonane	901	1.4
3.	α-Pinene	928	1.6
4.	Camphene	938	0.1
5.	β-Pinene	965	2.8
6.	β–Myrcene	989	0.1
7.	p-Cymene	1015	0.2
8.	β-Phellandrene	1019	0.3
9.	cis-Ocimene	1022	0.1
10.	Terpinen-4-ol	1147	1.6
11.	Estragole	1187	0.2
12.	Bornyl acetate	1273	0.1
13.	δ-Elemene	1338	0.2
14.	α-Copaene	1363	0.3
15.	β-Caryophyllene	1403	0.1
16.	α–*t*-Bergamotene	1436	0.4
17.	γ-Gurjunene	1450	0.2
18.	Valencene	1469	0.2
19.	γ-Curcumene	1470	0.5
20.	β-Selinene	1472	0.3
21.	α-Selinene	1475	0.2
22.	Ledol	1542	0.4
23.	*ar*-Turmerone	1615	3.0
24.	β-Eudesmol	1630	1.2
25.	Cadinol	1641	1.3
26.	Bisabolol oxide	1658	1.1
27.	Cinnamyl n-valerate	1705	0.2
28.	(2Z,6E)-Farnesol	1715	0.3
29.	Amyl anthranilate	1719	0.3
30.	Ethyl p-methoxycinnamate	1722	1.7
31.	Isoamyl pyruvate	1731	1.9
32.	Methyl heptadecane	1762	0.5
33.	n-Pentadecanol	1776	0.3
34.	n-Octadecane	1803	10.1
35.	(2Z,6E)-Farnesyl acetate	1824	0.2
36.	*n*-Octadecanol	1828	0.3
37.	Benzyl benzoate	1866	2.9
38.	n-Hexadecanol	1875	0.3
39.	Cinnamyl cinnamate	2056	48.9

Table 2: Antimicrobial activity of ethanolic extract of *C. oligantha var. lutea* rhizomes

Sample code	Sample conc. (µg)	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *E. coli*
A	200	24	30
B	100	21	29
C	50	18	26
Co	Control	00	00
S	Standard	21	21

Table 3: Antimicrobial activity of volatile oil of *C. oligantha* var. *lutea* rhizomes

Sample code	Sample conc. (µg)	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *E. coli*
A	200	20	21
B	100	18	20
C	50	14	17
Co	Control	00	00
S	Standard	21	20

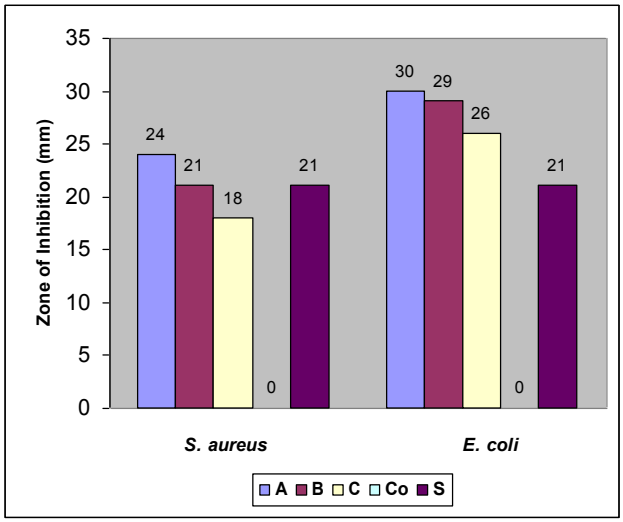

Figure 1: Antimicrobial activity of ethanolic extract of *C. oligantha* var. *lutea*

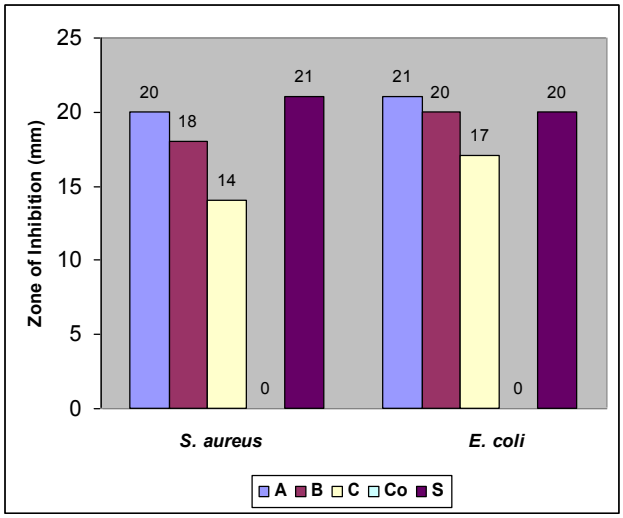

Figure 2: Antimicrobial activity of volatile oil of *C. oligantha* var. *lutea*

RESULTS AND DISCUSSION

The rhizomes of *C. oligantha* var. *lutea* on steam distillation yielded 3.2 % pale greenish yellow oil. The volatile components of *C. oligantha* var. *lutea*, percentage composition of each component and their retention indices are listed in Table 1. The constituents were arranged in order of GC and GLC elution on ULBON HR-1 fused silica capillary and Silicon DB-1 fused silica columns, respectively. Analysis of the oil by GC and GC-MS resulted in the identification of 39 components. Quantitatively, the oil was characterized by high amount of cinnamyl cinnamate (48.9%) followed by n-hexanal (14.0%) and n-octadecane (10.1%). The oil contained ten monoterpenes which amounted to 7.1% comprising seven monoterpene hydrocarbons (5.2%), one alcohol (1.6%) and one ester (0.1%). The predominant monoterpenes were β-pinene (2.8%), α-pinene (1.6%) and terpinen-4-ol (1.6%). Among 16 sesquiterpenes, constituting 9.7% of the oil, there were nine hydrocarbons (2.4%), five alcohols (4.3%), one ketone (3.0%) and one ester (0.2%). The major sesquiterpenes were ar-turmerone (3.0%), cadinol (1.3%), β-eudesmol (1.2%) and bisabolol oxide (1.1%). The prominent aromatic constituents included five esters (54.0%); the major esters were cinnamyl cinnamate (48.9%), benzyl benzoate (2.9%) and ethyl p-methoxy cinnamate (1.7%). Eight aliphatic constituents constituting 28.8% of the volatile oil comprised mainly n-hexanal (14.0%), n-octadecane (10.1%) and isoamyl pyruvate (1.9%).

The ethanolic extracts and volatile oils of the rhizomes of *C. oligantha* var. *lutea* were examined for antimicrobial activity against Gram positive (*S. aureus*) and Gram negative (*E. coli*) micro-organisms by cup-plate method. The sample extracts and the volatile oils showed significant antimicrobial activity when compared with the standard Aimikacin (Figure 1 and 2). The observations are shown in the Tables 2 and 3.

CONCLUSION

The manuscript describes volatile oil composition and antimicrobial activity of the rhizomes of *Curcuma oligantha* var. *lutea* for the first time.

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