AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Volatile oil Composition and Antimicrobial Activity of *Curcuma oligantha* var. *oligantha* Rhizomes

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ABSTRACT

Hydrodistilled volatile oil obtained from the rhizomes of *Curcuma oligantha* Trimen var. *oligantha* (Zingiberaceae) was analyzed by GC and GC-MS. The volatile oil was composed mainly of cinnamyl cinnamate (88.0 %), linalool (2.9 %) and α-phellandrene (2,2 %). Among the nine monoterpenes (6.9 %) present in the oil, the prominent ones were linalool (2.9%) and α-phellandrene (2.2 %). The oil contained ten sesquiterpenes (2.8 %) comprising mainly (2Z,6E)-farnesyl acetate (1.2%). The aliphatic constituents (2.5 %) were characterized as *n*-heptadecane, *n*-octadecanal, *n*-hexadecanol (1.4 %) and *n*- hexadecanoic acid. About 15 components occurred in trace amounts in the oil. The volatile oil and ethanolic extract of the rhizomes showed significant antimicrobial activity.

Keywords: *Curcuma oligantha* var. *oligantha*, Rhizomes, Volatile oil composition, Antimicrobial activity.

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Received 26 December 2012, Accepted 21 January 2013

Please cite this article in press as Ahmed S *et al.*, Volatile oil Composition and Antimicrobial Activity of *Curcuma oligantha* var. *oligantha* Rhizomes. American Journal of PharmTech Research 2013.

INTRODUCTION

Curcuma oligantha Trimen var. *oligantha* (Zingiberaceae) is an erect perennial herb with white flowers spotted yellow at the throat of the labellum. It is distributed in Karnataka and Sri Lanka [1,2]. The curcuma rhizomes are large, tuberous, orange-red and aromatic and substituted for turmeric. The rhizomes are prescribed to treat bruises, sprains, skin eruptions, infections and to improve complexion[3]. Oliganthyl cinnamate, curoliganthol, aliphatic constituents, curcumin, stigmasterol and β-sitosterol are reported from *C. oligantha* var. *oligantha* rhizomes[4.5]. This paper describes isolation and chemical composition of the volatile oil and antimicrobial activity of the rhizomes of *C. oligantha* var. *oligantha*.

MATERIALS AND METHODS

Plant Material

The rhizomes of *C. oligantha* var. *oligantha* (3 kg) 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 Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.

Preparation of ethanolic extract

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

Extraction of volatile oil

The fresh rhizomes of *C. oligantha* var. *oligantha* (1 kg) were hydrodistilled using an all glass Clavenger apparatus. A pale greenish yellow essential oil (3.82 %) 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 were 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[6-11]. 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 were 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 was obtained from the Department of Microbiology, Majeedia Hospital, New Delhi.

Media

Dehydrated nutrient agar media was prepared in distilled deionized water. The media (g/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 non-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 is 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 oil of *C. oligantha* var. *oligantha* were made having concentration of 200 µg, 100 µg and 50 µg / 0.1 ml of solution. Aimikacin discs 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. *oligantha* extract was used as test solution. Each group contains 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 hr 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.

RESULTS AND DISCUSSION

Steam distillation of *C. oligantha* var. *oligantha* rhizomes gave essential oil in the yield of 3.8 %. The resultant isolate on appropriate dilution possessed the characteristic odour of *C. oligantha*. Analysis of the oil by GC and GC-MS resulted in the identification of 25 components. The main contributor to the odour was found to be cinnamyl cinnamate (88%). Among the nine monoterpenes (6.9 %) present in the oil, the prominent ones were linalool (2.9%) and α-phellandrene (2.2 %). There were five monoterpene hydrocarbons (3.3 %), two alcohols and one each aldehyde and ketone. The oil contained ten sesquiterpenes (2.8 %) comprising mainly (2Z,6E)-farnesyl acetate (1.2%). The sesquiterpenes were composed of four each hydrocarbons and alcohols and one each of ketone and ester. The aliphatic constituents (2.5 %) were characterized as *n*-heptadecane, *n*-octadecanal, *n*-hexadecanol (1.4 %) and *n*- hexadecanoic acid. About 15 components occurred in trace amounts in the oil (Table 1).

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

S. No.	Components	RI	Percentage
1.	α-Thujene	925	0.5
2.	Sabinene	965	0.1
3.	α-Phellandrene	987	2.2
4.	*p*-Cymene	1009	0.1
5.	β-Phellandrene	1011	0.4
6.	Linalool	1081	2.9
7.	α-Terpien-4-ol	1160	0.4
8.	Neral	1237	0.1
9.	Pulegone	1259	0.2
10.	β-Caryophyllene	1403	0.1
11.	α-*t*- Bergamotene	1436	0.2
12.	Valencene	1471	0.2
13.	Ledol	1544	0.1
14.	α-Selinene	1619	0.1
15.	Caryophyllenol	1635	0.1
16.	ar-Turmerone	1639	0.3
17.	α-Bisabolol	1680	0.2
18.	β-Bisabolol	1688	0.1

19.	*n*-Heptadecane	-	0.2
20.	(2Z,6E)-Farnesyl acetate	1824	1.2
21.	*n*-Octadecanal	1829	0.3
22.	*n*-Hexadecanol	1879	1.4
23.	Hexadecanoic acid	1937	0.6
24.	Geranyl benzoate	1948	0.2
25.	Cinnamyl cinnamate	2055	88.0

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

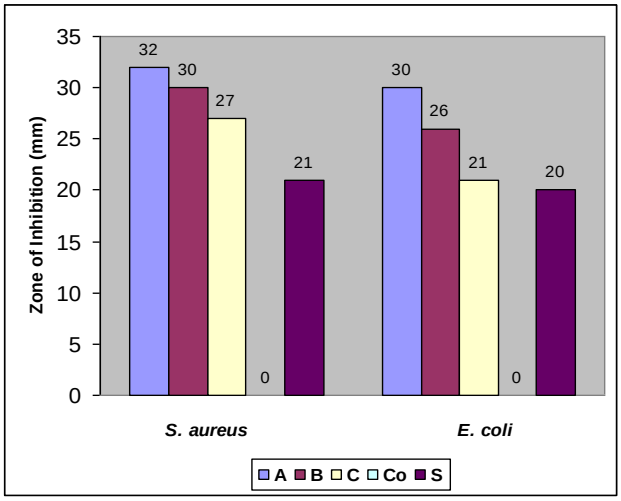

Figure 1. Antimicrobial activity of ethanolic extract of *C. oligantha* var. *oligantha.*

Co (control), S (standard), A (200 µg / 0.1 ml), B (100 µg / 0.1 ml) and C (50 µg / 0.1 ml)

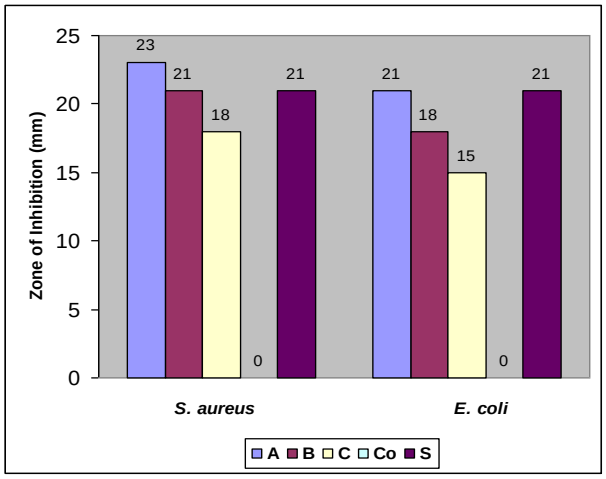

Figure 2. Antimicrobial activity of volatile oil of *C. oligantha* var *oligantha.*

Co (control), S (standard), A (200 µg / 0.1 ml), B (100 µg / 0.1 ml) and C (50 µg / 0.1 ml)

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

Sample code	Sample conc. (µg)	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *E. coli*
A	200	32	30
B	100	30	26
C	50	27	21
Co	Control	00	00
S	Standard	21	20

Co (control), S (standard), A (200 µg / 0.1 ml), B (100 µg / 0.1 ml) and C (50 µg / 0.1 ml)

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

Sample code	Sample conc. (µg)	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *E. coli*
A	200	23	21
B	100	21	18
C	50	18	15
Co	Control	00	00
S	Standard	21	21

Co (control), S (standard), A (200 µg / 0.1 ml), B (100 µg / 0.1 ml) and C (50 µg / 0.1 ml)

CONCLUSION

The volatile oil obtained from the rhizomes of *Curcuma oligantha* var. *oligantha* procured from Udupi, Karnataka was composed mainly of cinnamyl cinnamate (88.0%). The volatile oil and the ethanolic extract of the rhizomes showed significant antimicrobial activity.

ACKNOWLEDGEMENT

The authors are thankful to the Head, SAIF, Central Drug Research Institute, Lucknow for recording GLC and GC-MS analysis of the volatile oil.

REFERENCES

1. Ansari R, Nair VJ, Nair NC. Two new texa of *Curcumin* Linn. (Zingiberaceae) from Cannanore district, Kerala India. Curr.Sci1982; 51 (6): 293-294.
2. Bhat KG. Curcuma oligantha Trimen (Zingiberaceae) – a new record for India. Indian J. Forestry.1987; 10 (1): 66-68.
3. Kiritikar KR, Basu BD. Indian Medicinal Plants. Sri Satguru Publications, Delhi, 2000; 11: 3335.
4. Ahmad S, Ali M, Ansari SH. New cinnamyl ester and olyalcohol derivative from the rhizome of *Curcuma oligantha* var. *oligantha*, Acta Poloniac Pharma-Drug Research, 2010; 67: 563-566.
5. Ahmad S, Ali M, Ansari SH. New aliphatic constituents from the rhizomes of C*urcuma*

oligantha Trimen var. *oligantha* , Intern. J. Res. Ayurveda and Pharm 2011; 2:797-800.

6. Jennings WS, Adams RP. Identification of essential oil components by gas Chromatography/Qudrupol mass spectrometry. Allured publication Corp. Carol Stream, Illinois. 2001.
7. Shibamoto T. Qualitative Analysis of Flavour and Fragrance Volatiles by Capillary Gas Chromatography. Academic Press: New York, 1980.
8. Swiger AA, Silverstein RM. Monoterpenes. Aldrich Chemical Co: Milwaukee, WI. 1981.
9. M Ali. Techniques in Terpenoid Identification. Birla Publications, Delhi. 2001.
10. Joulain D, Konig W. The Atlas of spectral data of sesquiterpenes hydrocarbons, E.B. Verlag, Hamburg, Germany. 1998.
11. McLafferty FW. The Wiley Registry of Mass Spectral Data, 6th edition New York, John Wiley and Sons, 1994.