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Volatile oil composition and antimicrobial activity of *Curcuma aromatica* Salisb. rhizomes

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ABSTRACT

Hydrodistilled volatile oil obtained from the rhizomes of *Curcuma aromatica* Salisb. (Zingiberaceae) analyzed by GC and GC-MS was composed mainly of ar-turmerone (58.9%) followed by bicyclogermacrene (11.7%), (E)-2-hexenal (5.3 %), γ-himachalene (4.0 %), its isomer (3.8 %), caryophyllene oxide (3.4 %) and valencene (3.2 %). Among 16 sesquiterpenes consisting 91.6 %, the prominent sesquiterpenes were ar-turmerone (58.9 %), bicyclogermacrene (11.7 %) and γ- himachalene (4.0 %). Total five monoterpenes (3.3 %) were present in the oil including four hydrocarbons (2.3 %) and one alcohol (1.0 %). About 13 components occurred in trace amounts in the oil. The volatile oil and ethanolic extract of the rhizomes showed significant antimicrobial activity.

KEYWORDS

Curcuma aromatica;
Rhizomes;
Volatile oil composition;
Antimicrobial activity.

INTRODUCTION

Curcuma aromatica Salisb. (Zingiberaceae), commonly known as jangli haldi or yellow zedoary, is an erect perennial herb scattered throughout India and cultivated in West Bengal and Kerala. The rhizome is tuberous, large, orange-red and aromatic; substituted for turmeric and widely used as analgesic, sedative, hepatitis and anti-inflammatory. It is well known as a condiment, flavouring spice and an ingredient in skin cosmetics and applied externally to cure bruises, pain, inflammation, sprains, skin eruptions, infections and to improve complexion[1,2]. It has been reported that the essential oil or extracts from the rhizome of *C. aromatica* also possessed anti-inflammatory activity[3,4] along with antitumor and pharmacological effects[5] and antioxidant action[6-8]. The rhizomes contained zederone[9],9-oxo-neoprocurcumenol[10,11], neoprocu rcumenol[11], curcumin[12-14] and volatile oil mainly composed of β-curcumene, ar-curcumene, xanthorrhizol, germacrone, camphor, curzerenone, 7-methanoazulene, 1,8-cineole, β-elemene and linalool[7,15,16]. The present paper describes chemical composition of the volatile oil and antimicrobial activity of the ethanolic extract and volatile oil isolated from the rhizomes procured from Delhi.

MATERIAL AND METHODS

Plant material

The rhizomes of C. aromatica (3 kg) were obtained

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from Khari Baoli market, Delhi and identified by Prof. M.P.Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen was deposited in the herbarium of the Faculty of Pharmacy,

Extraction and isolation of volatile oils

The fresh rhizomes of *C. aromatica* (1 kg) were hydrodistilled using an all glass apparatus (Clavenger apparatus). A greenish brown essential oil (4.6 %) was obtained, dried over anhydrous sodium sulfate and stored at 4°C in the dark.

GC analysis

The GC analysis of the oil was performed on a Varian 3300 gas chromatogram, using a fused capillary column (30 M x 0.25 mm internal diameter, film thickness 0.25 m), coated with dimethyl siloxane (DB-1). The oven temperature was programmed at 80-225°C at 4°C / min, then held isothermal at 250°C. Detector used FID, detector temperature 300°C, injection volume 0.1 ml and carrier gas nitrogen was used.

GC-MS analysis

GC-MS data were obtained on a Shimadzu QP 2000 instrument at 70 eV and 250°C. GC column : ULBON HR-1, equivalent to OV-1, fused silica capillary-0.25 mm x 50 M with film thickness- 0.25 m. The initial temperature was 100°C for 6 minutes and then heated at the rate of 10°C / minute to 250°C. Carrier gas, helium, at a flow rate of 2 ml / minute was used.

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[17-21]. 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.

TABLE 1 : Chemical composition of the volatile oil of *C. aromatica* rhizomes.

S. No.	Components	RI	Percentage
1.	(E)-hexanal	827	5.3
2.	β-Myrcene	971	0.5
3.	α-Phellandrene	1000	0.8
4.	*p*-Cymene	1005	0.8
5.	1,8-Cineole	1017	1.0
6.	(Z)-β-Ocimene	1028	0.2
7.	*t*-β-Caryophyllene	1403	0.2
8.	γ- Curcumene	1475	0.4
9.	Valencene	1477	3.2
10.	γ-Himachalene	1479	4.0
11.	Z-α-Bisabolene	1490	0.9
12.	Caryophyllene oxide	1548	3.4
13.	Globulal	1572	0.4
14.	γ-Himachalene isomer	1578	3.8
15.	Epiglobulol	1582	0.5
16.	*ar*-Turmerone	1611	58.9
17.	Bicyclogermacrene	1615	11.7
18.	Ledene epoxide	1621	0.3
19.	Caryophyllenol	1635	0.9
20.	α-Bisabolol	1645	1.9
21.	β-Bisabolol	1649	0.3
22.	Viridiflorol	1737	0.8

Antimicrobial activity

Test organisms and inoculums

Escherichia coli (NCTC-6571) and *Staphylococcus aureus* (NCTC-10418) were obtained from the Department of Microbiology, Majeedia Hospital, New Delhi.

Standard

Aimikacin discs with specific activity of 30 mg was obtained from the Department of Microbiology, Majedia Hospital, New Delhi.

Media

Dehydrated nutrient agar media was used and was prepared in distilled deionized water. The media (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

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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 bung. 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.

Methods of 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 37^0C 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 inoculum and during inoculation in a plate assay.

Methods : 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. aromatica* were made having concentration of 200 mg, 100 mg and 50 mg / 0.1 ml of solution. Aimikacin discs of 30 mg concentration was used as standard (S). The plates were labelled as Co (control), S (standard), A (200 mg / 0.1 ml), B (100 g / 0.1 ml) and C (50 mg / 0.1 ml) corresponding to different holes. 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^oC. After 24 hours the plates were examined and the diameter of zones of inhibition was accurately measured.

RESULTS AND DISCUSSION

Hydrodistilled volatile oil obtained from the rhizomes of *C. aromatica* was analyzed by GC and GC-MS. The oil composition is summarized in TABLE 1. The components are arranged in order of GC elution on silicon DB column. About 21 constituents (96.2 %) were positively detected in the volatile oil. The prominent component was ar-turmerone (58.9 %) followed by bicyclogermacrene (11.7 %), (E)-2-hexenal (5.3 %), β-himachalene (4.0 %), its isomer (3.8 %), caryophyllene oxide (3.4 %) and valencene (3.2 %). The oil was characterized by a large amount of sesquiterpenes. Among 16 sesquiterpenes, consisting 91.6 %, there were seven hydrocarbons (24.2 %), five alcohols (4.4 %), one aldehyde (0.4 %), one ketone (58.9 %), and two oxides (3.7 %). The prominent sesquiterpenes were ar-turmerone (58.9 %) bicyclogermacrene (11.7 %) and ϒ-himachalene (4.0 %). Among five monoterpenes (3.3 %) present in the oil, there were four hydrocarbons (2.3 %) and one alcohol (1.0 %). p-Cymene (0.8%) and ã-curcumene (0.4 %) were characterized as the aromatic ring containing compounds present in the oil of *C. aromatica*. (E)-2-Hexenal (5.3 %) was the only aliphatic constituent present in the volatile oil. One of the earlier report indicated that germacrene-D, curzerenone, xanthorrhizol, curcuphenol and hydroxyisogermafurenolide were the most notable constituents[22]. The essential oil of *C. aromatica* from north

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east India possessed predominantly camphor (32.5 %), curzerenone (11.0 %), β-turmerone (6.7 %) and 1,8-cineole (5.5 %)[23]. The essential oil of *C. aromatica* from Taiwan is composed mainly ar-turmerone (49.0 %), humulene oide (16.6 %) and β-selinene (10.2 %)[6]. The major constituents detected in the volatile oil of *C. aromatica* grown in Thailand were ar-curcumene (23.2 %) and xanthorrhizol (18.7 %)[24] and camphor (7.3 %) and ar-curcumene (5.6 %)[25]. A report from China indicated the presence of curdione, neocurdione, curcumol, tetramethyl pyrazine and 1,2-hexa decanediol[26]. The environmental factors may be responsible for this variation of chemical constituents in the volatile oil of *C. aromatica*.

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

TABLE 2 : Antimicrobial activity of ethanolic extract of *C. aromatica* rhizomes.

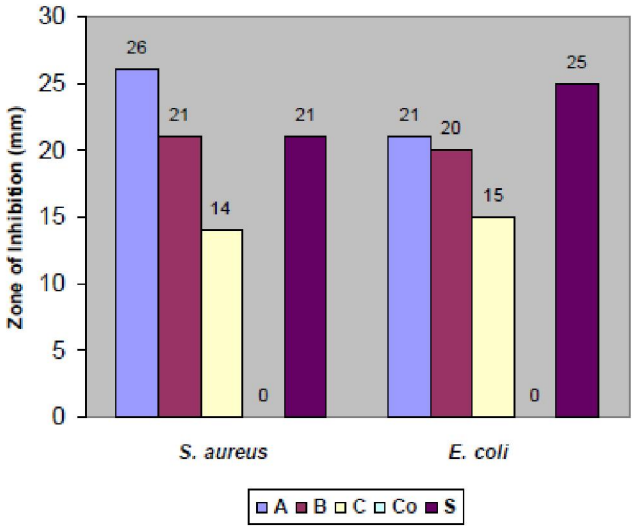
Sample code	Sample conc.(µg)	Zone of inhibition (mm)against *S.aureus*	Zone of inhibition (mm)against *E.coli*
A	200	26	21
B	100	21	20
C	50	14	15
Co	Control	00	00
S	Standard	21	25

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. aromatica* rhizomes.

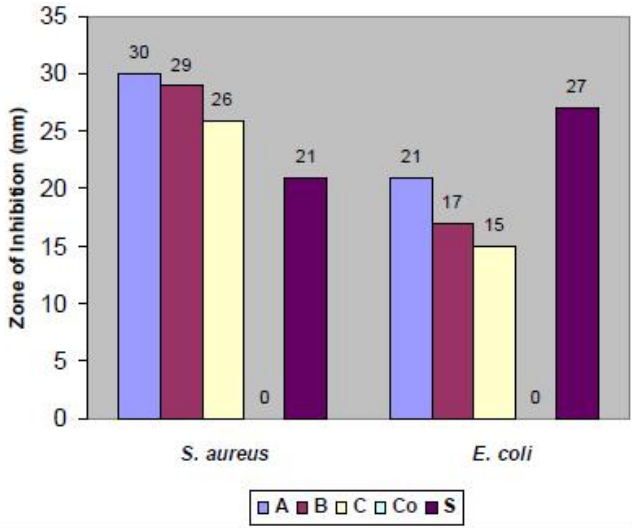
Sample code	Sample conc.(µg)	Zone of inhibition (mm)against *S.aureus*	Zone of inhibition (mm)against *E. coli*
A	200	30	21
B	100	29	17
C	50	26	15
Co	Control	00	00
S	Standard	21	27

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



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

Figure 1 : Antimicrobial activity of ethanolic extract of *C. aromatica*.



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

Figure 2 : Antimicrobial activity of volatile oil of *C. aromatica*.

CONCLUSIONS

The volatile oil of the rhizomes of *Curcuma aromatica* Salisb. (Zingiberaceae) of Delhi origin was composed mainly of ar-turmerone (58.9%), bicyclogermacrene (11.7%), (E)-2-hexenal (5.3 %), ϒ-himachalene (4.0 %), its isomer (3.8 %), caryophyllene oxide (3.4 %) and valencene (3.2). The volatile oil and the ethanolic extract of the galls showed significant antimicrobial activity.

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