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Volatile constituents of essential oil of *Curcuma aromatica* salisb

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ABSTRACT

Essential oil from a new chemotype of C.*aromatica* Salisb. collected from the high altitude region of Northern India was analysed by GC FID and GC-MS on polar and non polar columns. On polar column, thirty-five constituents were identified in the essential oil of rhizomes representing 92.94% identification and thirty-nine constituents were identified in the essential oil of its leaves representing 97.99% identifications. The major constituents in the rhizome oil were identified as camphor (27.50%), 1,8 cineole (12.39%) and curdione (10.66%) and 1, 8 cineole (34.01%), camphor (13.94%), α-terpineol (6.31%) and α-terpinolene (5.04%) in the leaf oil. Analysis on nonpolar column resulted in identification of thirty-one constituents in the rhizome oil representing 86.70% identifications and twenty-nine constituents identified in the leaf oil representing 94.44% identification. The major constituents were camphor (17.86%), 1, 8 cineole (14.95%), isoborneol (10.70%) and borneol (6.64%) in the rhizome oil and α-pinene (6.06%), camphene (7.49%), 1,8 cineole (21.87%), camphor (11.75%), and isoborneol (6.44%) in the leaf oil.

KEYWORDS

Curcuma aromatica;
Zingiberaceae;
Essential oil;
GC;
GC-MS;
1,8 Cineole;
Camphor;
Curzerene;
Chemotype.

INTRODUCTION

Genus *Curcuma* Salisb. belongs to family Zingiberaceace and consists of 70 species of rhizomatous herbs distributed throughout tropical and subtropical regions of the world especially in Indonesia, Malaysia, Thailand and India. About 30 species are found in India of which few are of economic importance[7] and used in traditional system of medicine[2]. Rhizomes of this plant are a source of essential oil useful in perfumery compounds and also having medicinal importance[4,5,15,16,11]. Many species of this genus are used in foods, dyes and in traditional Chinese, Japanese and Indian system of medicine[7,8]. Some species are used as antiheptotozal, anti-inflammatory, in bile expulsion, anti-ulcer, antimicrobial, stomachic, insecticidal and as antiprotozoal[7,13]. Natural curcuminoids reported from *C.longa* have anti-oxidant properties[12]. The major sesquiterpenes including germacrone, curdione, neocurdione, curcumenol and curcumin, are reported to have protective effect on Dgalactosamine/lipopolysaccharide-induced liver injury in mice[9].

Essential oil of *C.aromatica* is reported to have insecticidal properties against Odontotermes obesus Rhamb, a pest of sugar cane[14]. Earlier chemical composition of *C.aromatica* oil is reported to contain p-cymene (25.2%), 1,8 cineole (24.0%), p-cymene-8-ol (4.6%), ar-turmerol (3.8%) and α-terpineol (8.1%) in its leaf oil as mojor constituents[14,3]. However, in the leaf oil our findings did not identify ar-turmerol and pcymene-8-ol but instead a characteristic sesquiterpene hydrocarbon curzerene an important constituent of this oil was identified which has not been reported earlier in this species and categorises this plant as a new chemotype. However, this constituent is reported[13] in another species, *C.Zedoaria* alongwith its oxygenated sesquiterpenes curzerenone and epicurzerenone. In view of our findings, this differed significantly from the previous reports, led to 3 the identification of a new chemotype of *C.aromatica* with curzerene as one of the major constituents.

MATERIAL AND METHODS

Plant material

The rhizomes and leaves of cultivated plant material of *C.aromatica* were collected from Pauri Garhwal region of Uttarakhand, during Oct 2007 located at an altitude of 1300m above mean sea level.

Isolation of essential oil

For quantitative analysis air dried rhizomes and leaves were separately taken and cut into small pieces, washed with distilled water and subjected to hydrodistillation in a Clevenger-type apparatus for 6h. The oil samples obtained were dried over anhydrous sodium sulphate and stored in sealed glass viles in a refrigerator at 4-5^{0}C prior to analysis. The yield of the oil was 0.40% v/w and 0.45% v/w in rhizomes and leaves respectively on dry weight basis.

Gas chromatography-Mass Spectrometry (GC-MS)

GC analysis was performed on Shimadzu GC-2010 gas chromatograph coupled to Shimadzu CR-3A data processor system and fitted with a 30m $\times$ 0.25mm $\times$ 0.25μm BP-20 column and 30m $\times$ 0.25mm $\times$ 0.25 μm DB-5 columns, temp prog: initial temp. 45^{0}C for 2 min @ 50/min, final temp. 250^{0}C for 12 min. injector temp: 250^{0}C and detector temp 200^{0}C with carrier gas nitrogen, split injection mode purge flow 1ml/min, split ratio 50.0. and GC-MS spectra were recorded on Shimadzu QP 2010 (70ev) fitted with fused silica capillary column 25 m $\times$ 0.25mm, 0.25 μm, BP-20 column and 25 m $\times$ 0.25m, 0.25 μm DB-5 column, carrier gas helium, ion source temp 2000C, interface temp 250^{0}C. The column conditions used were same as in GC.

Identification of components

The identification of constituents was made on the basis of comparison of their mass fragmentation pattern with the corresponding data of authentic compounds available in the Literature[10,6] Relative percentage amounts of the separated compounds was calculated automatically from the peak areas of the total ion chromatogram (TIC). Further confirmation of the constituents was achieved by Kovats retention index (KRI) data generated against series of n-alkanes. (C8-C27) used as reference point in the calculation of relative retention indices. The composition of the essential oil of rhizomes and leaves on polar and non-polar column is given in TABLE 1.

RESULTS AND DISCUSSION

The essential oil was obtained by hydrodistillation of rhizomes and leaves of cultivated *C.aromatica* plants in 0.40% and 0.45% yields (v/w), respectively on fresh weight basis. GC-MS analysis of leaf oil on polar column resulted in the identification of 39 constituents representing 97.99% identifications. The non-terpene category represented 2-propanone 1.01%, 2-propanol 0.84% and ethanol 0.50%. Major monoterpene hydrocarbons were represented by α- pinene 3.67%, β-pinene 0.64%, camphene 4.83% and terpinolene 5.04%, major oxygenated monoterpenes were identified as 1,8 cineole 34.01%, isoborneol 4.98%, camphor 13.94%, α-terpineol 6.31% and major

sesquiterpene hydrocarbons represented β-elemene 1.24%, germacrene-A 0.89%, germacrene-B 0.44%, germacrene-D 0.15% and major oxygenated sesquiterpenes represented isospathulenol 0.33%, curdione 0.96% and neocurdione 3.73%. GC-MS analysis of rhizome oil resulted in the identification of 35 compounds representing 92.94% identifications in which non-terpenes identified are tricyclene 0.18%, heptanol 0.13% and valeric acid 0.37%. Major monoterpene hydrocarbons represented α-pinene 0.52%, β-pinene 0.15%, camphene 3.94%, β-myrcene 0.51%, α-terpinene 0.47%, limonene 1.20% and terpinolene 4.14 %. Major oxygenated monoterpenes 5 represented 1,8 cineole 12.39%, borneol 5.70%, camphor 27.5%, α-terpinolene 4.14% and isoborneol 8.99%. Major ses-

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TABLE 1: Chemical composition of the volatile oils from the rhizomes and leaves of *curcuma aromatica*

BP-20 column								DB-5 Column								
Rhizomes				Leaves				Rhizomes				Leaves				Identification
Peak no.	Compounds	RI	% Area	Peak no.	Compounds	RI	% Area	Peak no.	Compounds	RI	% Area	Peak no.	Compounds	RI	% Area	
1	Tricyclene	1007	0.18	1	2-Propanone	810	1.01	1	Tricyclene	301	0.32	2	2-Propanone	-	2.08	GC-MS
2	α-Pinene	1039	0.52	2	2-Propanol	884	0.84	2	α-Pinene	319	1.11	3	Tricyclene	301	0.37	GC-MS
3	Camphene	1083	3.94	3	Ethanol	1002	0.50	3	Camphene	340	6.87	4	α-Pinene	319	6.06	GC-MS
4	β-Pinene	1124	0.15	5	α-Pinene	1039	3.67	4	Sabinene	379	0.19	5	Camphene	340	7.49	GC-MS
5	β-Myrcene	1156	0.51	6	Camphene	1083	4.83	5	β-Pinene	386	0.30	6	Sabinene	379	1.79	GC-MS
6	Limonene	1206	1.20	7	β-Pinene	1124	0.64	6	β-Myrcene	408	0.45	7	β-Pinene	386	1.12	GC-MS
7	1,8 Cineole	1228	12.3	9	8 Sabinene	1130	1.22	7	1,8 Cineole	485	14.95	8	β-Myrcene	408	1.76	GC-MS
8	Heptanol	1284	0.13	9	β-Myrcene	1156	1.30	8	α-Terpinolene	608	5.20	9	1,8 Cineole	485	21.87	GC-MS
9	Terpinolene	1287	0.24	10	1,8 Cineole	1228	34.01	9	Valproic acid	0.21		10	α-Terpinolene	608	6.08	GC-MS
10	Camphor	1518	27.50	11	p-Cymene	1228	0.21	10	Camphor	734	17.86	11	Valproic acid	0.68		GC-MS
11	α-Terpinolene	1631	4.14	12	2- Heptanol	1284	0.18	11	Isoborneol	764	10.70	12	Camphor	734	11.75	GC-MS
12	β-Elemene	-	1.38	13	*Ttrans* Sabinene hydrate	-	0.20	12	Borneol	789	6.64	13	Camphenehydrate	746	3.18	GC-MS
13	Camphene hydrate	-	0.40	14	Camphor	1518	13.94	13	Terpine-4-ol	820	0.74	14	Isoborneol	764	6.44	GC-MS
14	Terpinene	1661	0.47	15	α-Terpinolene	1287	5.04	15	δ-Elemene	1236	0.85	15	Borneol	789	5.38	GC-MS
15	β-Selinene	-	0.41	16	β-Elemene	-	1.24	16	β -Elemene	1375	0.22	16	Terpinene-4-ol	820	0.86	GC-MS
16	Isoborneol	1660	8.99	17	Camphene hydrate	-	1.59	18	Caryophyllene	1409	0.35	17	α-terpineol	852	3.79	GC-MS
17	γ-Muurolene	1727	0.62	18	Terpineol-4-ol	1631	0.59	19	Bicyclogermacrene	1632	0.44	19	β-Elemene	1375	2.92	GC-MS
18	Borneol	1698	5.70	19	β-Selinene	-	0.88	20	Aromadendrene	1491	0.16	20	Caryophyllene	1442	0.33	GC-MS
19	Carvone	1715	0.14	20	Isoborneol	1660	4.98	-	-	-	-	21	Germacrene-D	1594	0.27	GC-MS
20	Germacrene-A	-	1.03	21	β- Selinene	1608	0.96	22	Muurolene	1586	0.26	22	β-Selinene	1608	1.63	GC-MS
21	Germacrene-B	1777	0.57	22	Germacrene-D	1716	0.15	23	Germacrene-D	1594	0.84	24	Curzerene	1635	4.00	GC-MS
22	Curzerene -	-	1.70	23	α -Terpineol	1661	6.31	25	Curzerene	1635	5.51	25	Germacrene-B	1777	0.25	GC-MS
23	*Cis*-Muurol-5-en-4-α-ol	-	0.16	24	Germacrene-A	-	0.89	26	δ-Cadinene	1700	0.32	26	Spathulanol	1825	0.32	GC-MS
24	Valeric acid	-	0.37	25	Germacrene-B	1777	0.44	27	Germacrene-B	1777	0.35	27	*cis* -β-Elemone	1871	0.92	GC-MS
25	Spathulenol	2151	0.30	26	Curzerene	1635	1.30	28	Globulol	1841	0.61	28	Humulene epoxide	1897	0.33	GC-MS
26	Germacrone	-	0.32	27	Germacrene-D-4-ol	1821	0.14	29	*cis* β-Elemone	1871	1.77	29	Cubenol	1977	0.19	GC-MS
27	Furanodiene		0.81	28	Valeric acid		1.06	32	epi-α- Cadinol	1973	0.32	31	Curcuminol	2167	0.46	GC-MS
28	*cis*- β-Elemone	- 4.04		30	Carryophyllene acetate	2113	0.20	33	Germacrone	2097	2.16	32	Germacrone	2097	0.61	GC-MS
29	Selin-11-en-4-α-ol	-	0.15	32	*cis*-Humuleneoxide	1967	0.38	34	Curdione	-	4.51	33	Curdione	-	1.51	GC-MS
31	Curdione	-	10.66	33	β-Elemone	-	0.19	35	Neocurdione	-	2.28	-	-	-	-	GC-MS
32	Caryphylleneoxide		0.47	35	Germacrone	-	0.35	39	Valleral	1967	0.21	-	-	-	-	GC-MS
33	α-Muurolene	1730	0.57	37	Furanodiene	0.66	-	-	-	-	-	-	-	-	-	GC-MS
34	Curcumol	-	0.16	38	Curdione	0.96	-	-	-	-	-	-	-	-	-	GC-MS
35	Neocurdione	-	2.45	39	γ-Elemene	-	2.46	-	-	-	-	-	-	-	-	GC-MS
36	Docanoic acid methyl ester	-	0.17	40	Isospathulenol	0.33	-	-	-	-	-	-	-	-	-	GC-MS
-	-	-	-	41	Neocurdione	-	3.73	-	-	-	-	-	-	-	-	GC-MS
-	-	-	-	42	Curcumol	-	0.20	-	-	-	-	-	-	-	-	GC-MS
-	-	-	-	45	Docanoic acid methyl ester	-	0.26	-	-	-	-	-	-	-	-	GC-MS
-	-	-	-	46	Caryphyllene oxide	1967	0.15	-	-	-	-	-	-	-	-	GC-MS

quiterpene hydrocarbons represented β-elemene 1.38%, γ-muurolene 0.62%, germacrene-A 1.03%, germacrene-B 0.57% and major oxygenated sesquiterpenes represented spathulenol 0.30%, α-muurolene 0.57%, curdione 10.66% and germacrone 0.32%.

On carrying out GCMS analysis on non- polar column percentage variations in the volatile constituents in rhizome oil as well as in leaf oil was observed. Analysis on non-polar column resulted in the identification of 29 constituents in the leaf oil representing 94.44% identification in which non-terpenes represented 2-propanone 2.08%, tricyclene 0.37% and valproic acid 0.68%.

Monoterpene hydrocarbons represented α-pinene 6.06%, β-pinene 1.12%, camphene 7.49%, β-myrcene 1.76%, sabinene 1.79% and terpinolene 6.08 %. Oxygenated monoterpenes represented 1,8 cineole 21.87%, borneol 5.38%, camphor 11.75%, α-terpinolene 6.08%, isoborneol 6.44%, α-terpineol 3.79% and camphene hydrate 3.18%. Sesquiterpene hydrocarbons represented β-elemene 2.92%, caryophyllene 0.33%, β-Selinene 1.63%, germacrene-D 0.27%, germacrene-B 0.25% and oxygenated sesquiterpenes represented spathulenol 0.32%, humulene-epoxide 0.33%, curcuminol 0.46%, curdione 1.51%, germacrone 0.61% and curzerene 4.0%. However GC-MS analysis of rhizome oil resulted in the identification of 31 constituents representing 86.70% identifications in which non-terpenes represented tricyclene 0.32% and valproic acid 0.21%. Monoterpene hydrocarbons identified are α-pinene 1.11%, β-pinene 0.30%, camphene 6.87%, β-myrcene 0.45%, sabinene 0.19% and terpinolene 5.20 %. Oxygenated monoterpenes represented 1,8cineole 14.95%, borneol 6.64%, camphor 17.86%, isoborneol 10.70% and terpineol-4-ol 0.74%. Sesquiterpene hydrocarbons represented β-elemene 0.22%, γ-elemene 0.85%, caryophyllene 0.35%, germacrene-D 0.84%, germacrene-B 0.35%, bicyclogermacrene 0.44% and oxygenated sesquiterpenes represented curdione 4.51%, neocurdione 2.28%, germacrone 2.16%, curzerene 5.51%, globulol 0.61% as major constituents.

Analysis of leaf oil on polar column identified sabinene 1.22%, p-cymene 0.21% *trans*sabinene hydrate 0.20%, germacrene-A 0.89%, germacrene-D-4-ol 0.14% caryophyllene acetate 6 0.20%, *cis*-humuleneoxide 0.38%, β-elemene 0.19%, furanodiene 0.66%, γ-elemene 2.46%, neocurdione 3.73%, curcumol 0.20%, docanoic acid methyl ester 0.20% caryophyllene oxide 0.15% as additional constituents in comparision to analysis on non-polar column. Similarly analysis of rhizome oil on polar column identified limonene 1.20%, terpinolene 0.24%, camphene hydrate 0.40%, terpinene 0.47%, β-selinene 0.41%, carvone 0.14%, germacrene-A 1.03%, cis-muurol-5-en-4--ol 0.16%, spathulenol 0.30%, furanodiene 0.81%, selin-11-en-4-α-ol 0.15%, caryophyllene oxide 0.47%, α-muurolene 0.57%, curcumol 0.16%, dodecanoic acid methyl ester 0.17% while its analysis on non polar column identified sabinene 0.30%, d-elemene 0.85%, caryophyllene 0.35%, bicyclogermacrene 0.84%, d-cadinene 0.32%, globulol 0.61%, epi-α-cadinol 0.32% as additional constituents.

It is worth mentioning here that fresh sample of essential oil distilled from the rhizomes when analysed on polar column represented 1,8 cineole as major constituent. When analysis of the same oil sample was repeated on the same column after a few days, camphor was found as major constituent. So far this phenomenon of conversion of 1, 8 cineole into camphor is not reported in the literature of this genus. The colour of the fresh sample at the time of GC and GC-MS analysis was light blue and after few days the colour changed to light yellow despite keeping the sample in the refrigerater. However, to check the stability of this oil it needs further analysis of samples periodically to reach to some logical conclusion. In the light of present investigation 1,8 cineole, camphor, borneol, isoborneol and curzerene were found major constituents in both leaf and rhizome oils. In previous investigation[3] curzerene has not been reported in cultivated *C.aromatica* oil although the oils analysed by us are also distilled from the rhizomes and leaves of cultivated source. *C.aromatica* oil analysed by[14] is also devoid of curzerene but the authors here not mentioned whether the oil is extracted from natural or cultivated source. It is, therefore concluded that the present investigation has led to the identification of a new curzerene chemotype of *C.aromatica*.

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