

**CHEMICAL CHARACTERIZATION OF ESSENTIAL OIL FROM THE RHIZOMES OF *KAEMPFERIA ROTUNDA L.* BY GC/MS TECHNIQUE****AJAY KUMAR****Department of Applied Chemistry, Faculty of Engineering and Technology,
Gurukul Kangri University, Haridwar-249404 (U.K.), India***ABSTRACT**

The essence oil from the fresh rhizomes of *Kaempferia rotunda L.* was isolated by water distillation and analyzed by gas chromatography-mass spectrometry (GC/MS) method. Fifty seven components were identified in the rhizomes oil, which accounted to 99.74% of the oil. The major components were endo-borneol (9.30%), dehydro iso androsterone acetate (9.12%), naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl),[4aS-(4 α ,5 β ,8 α)]- (9.03%), and β -phellandrene (7.08%), other prominent constituents were 2-propenoic acid, 3-(3-methoxyphenyl)-, ethyl ester (4.47%), α -pinene (3.39%), ethyl cinnamate, trans (3.15%), 2,6-octadienoic acid, 3,7-dimethyl-, methyl ester (2.90%) and camphene (2.89%).

KEY WORDS: *Kaempferia rotunda L.*, Essential oil, GC/MS, Endo-borneol**AJAY KUMAR****Department of Applied Chemistry, Faculty of Engineering and Technology,
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INTRODUCTION

Kaempferia rotunda L. is a member of *Zingiberaceae* family which has been used as spice and as medicinal herb for thousands of years in India and South East Asia (Philip C. Stevenson et al., 2007; S. Sini et al., 2014). It is a stemless aromatic perennial herb mentioned in Ayurveda (Indian traditional system of medicine) named Hallakam or Bhumi-Champaka. The tuberous rhizomes of *Kaempferia rotunda* L. have been described in Ayurveda for treating a lot of human ailments including vata, kapha, mumps, wounds, blood clotting, cancer swellings, inflammations, stomachic problems, gastropathy, ulcer and diarrhoea colic disorder^{1,2,3}. The rhizomes of this plant are widely used in the folk medicines of Bangladesh for the treatment of high blood sugar and pain (Zakia Sultana et al., 2012). Despite these, the rhizomes are also used as food flavouring and in cosmetics in several Asian countries. Recent investigations revealed the antioxidant, anthelmintic, antihyperglycemic, antinociceptive, antimutagenic and strong antiplatelet

aggregation activities of the methanolic extracts of *Kaempferia rotunda* L. rhizomes^{4,5,6,7,8}. Recently, a new lactin has been purified from the rhizomes of this plant by S.R. Kabir et al., which showed antibacterial and antiproliferative activities⁹. The present study focused on the isolation and chemical characterization of essential oil from the rhizomes of *Kaempferia rotunda* L.

EXPERIMENTAL

Collection of Plant

The fresh plants of *Kaempferia rotunda* L. were collected from Sushila Tiwari herbal garden, Rishikesh, Uttarakhand, India. The plant was taxonomically identified by the staff of Botanical Survey of India and a voucher specimen (Acc. No. 114817) was deposited at the Herbarium of Botanical Survey of India, Dehradun, India. The plant is shown in Figure 1.



Figure 1
Kaempferia rotunda L.

Isolation of Essential Oil

100g of fresh and finely chopped rhizomes of *Kaempferia rotunda* L. were subjected to water distillation for 8 hours using a Clevenger-type apparatus¹⁰. The oil was extracted from the distillates with n-hexane and then dried over anhydrous sodium sulphate. Thereafter, the oil was stored in a fridge at 4°C until analysed.

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The Thermo Scientific Trace 1300 gas chromatograph coupled with TSQ 8000 mass spectrometer (triple quadruple), with impact ionization (EI) method, was used to determine the chemical composition of essential oil

isolated from the rhizomes of *Kaempferia rotunda* L. Thermo TG 5MS non polar fused silica capillary column (30 m length x 0.25mm diameter x 0.25 µm thickness) was used for the following conditions:

Oven Program: 50 °C hold for 2 min. to 280 °C at the rate of 10 °C/min. and hold it for 15 min.

Carrier gas= helium @ 1 ml/min.

Injection volume= 1 µL of diluted oil in n-hexane

Injector temperature= 250 °C

Transfer line temperature= 300 °C

MS source temperature= 280 °C

Ionization energy= 70 eV

Split injection ratio of 1:50

Scan mass range of m/z 50-1000 amu

Identification of Compounds

The individual components of the essential oil were identified by computerized matching of their mass spectra of peaks with those gathered in the NIST-Mass Spectral library of the GC/MS data software system.

RESULTS AND DISCUSSION

The essential oil of the fresh rhizomes of *Kaempferia rotunda* L. was obtained by water distillation with a yield of 0.034% and characterized by gas chromatography-mass spectroscopy (GC/MS) method for its chemical constituents. Fifty seven compounds were identified which are listed in Table 1.

Table 1
Chemical constituents of essential oil of *Kaempferia rotunda* L.

RT	% Area	Compound
5.96	0.90	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-
6.18	3.39	α-Pinene
6.45	2.89	Camphene
6.93	2.46	(-)-β-Pinene
7.12	1.78	β-Myrcene
7.36	0.44	β-Thujene
7.47	0.99	3-Carene
7.82	7.08	β-Phellandrene
8.94	0.61	Linalool
9.19	0.58	Bicyclo [2.2.1] heptane, 2-methoxy1,7,7 trimethyl
9.34	0.53	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-
9.46	0.38	1-Methylverbenol
9.65	0.42	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-
9.73	1.11	trans-3(10)-Caren-2-ol
10.13	9.30	endo-Borneol
10.27	1.16	(-)-Terpinen-4-ol
10.45	0.94	L-α-Terpineol
10.75	0.40	l-Verbenone
10.93	2.01	Citronellol
11.33	1.17	(S)-(-)-Citronellic acid, methyl ester
11.68	0.32	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-
11.80	2.33	Levo-Bornyl acetate
12.26	2.90	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester
13.25	0.93	β-Elemene
13.70	2.79	Caryophyllene
14.12	0.41	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
14.21	3.15	Ethyl cinnamate, trans
14.46	0.60	β-Cedrene
14.54	0.50	β-Selinene
14.64	0.85	α-Selinene
14.85	0.42	γ-Murolene
14.93	0.35	δ-Cadinene
15.76	1.26	Caryophyllene oxide
16.09	0.42	Cubenol
16.18	0.37	8-Epi-γ-gama.-Eudesmol
16.31	1.29	Isoledene
16.39	0.37	α-Acorenol
16.50	2.64	Ethyl p-methoxycinnamate
16.56	2.84	β- Eudesmol

16.75	0.47	(-)-Spathulenol
17.16	0.34	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol
17.34	0.82	Longipinocarveol, trans
17.63	4.47	2-Propenoic acid, 3-(3-methoxyphenyl)-, ethyl ester
18.22	0.33	2-(1H) Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl) ethenyl]-4,8a-dimethyl, [4aR-(4a α ,6 α ,8 α)]-
19.90	1.35	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-
21.34	2.36	Aristolene epoxide
21.49	1.50	Podocarp-7-en-3 β -ol, 13 β -methyl-13-vinyl-
21.61	1.84	5-Androsten-3,17-dione
21.74	0.84	Cedrene epoxide
22.00	9.12	Dehydroisoandrosterone acetate
22.09	0.85	Retinol acetate
22.25	0.29	Larixol acetate
22.84	9.03	Naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl), [4aS-(4 $\alpha\alpha$,5 β ,8 $\alpha\alpha$)]-
23.05	2.12	Pimaric acid
23.43	0.29	Verticiol
23.47	0.44	17 α -Hydroxypregnenolone
99.74%		Total Identified Constituents

RT= Retention Time on Thermo TG 5MS column; identification was done by NIST-MS Spectral library of the GC/MS data software system.

The results showed the major constituents identified were endo-borneol (9.30%), dehydroisoandrosterone acetate (9.12%), naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl), [4aS-(4 $\alpha\alpha$,5 β ,8 $\alpha\alpha$)]- (9.03%), β -phellandrene (7.08%), 2-Propenoic acid, 3-(3-methoxyphenyl)-, ethyl ester (4.47%), α -pinene (3.39%), ethyl cinnamate, trans (3.15%), 2,6-octadienoic acid, 3,7-dimethyl-, methyl ester (2.90%), camphene (2.89%), β - eudesmol (2.84%), caryophyllene (2.79%), ethyl p-methoxycinnamate (2.64%), (-)- β -pinene (2.46%), aristolene epoxide (2.36%), levo-bornyl acetate (2.33%), pimaric acid (2.12%) and citronellol (2.01%). Results showed that the main identified compounds are terpenoids, steroids and esters which have significant biological activities and accountable for the biological properties of the oil. Endo-borneol, β -phellandrene, camphene, β -eudesmol, caryophyllene and citronellol are medicinally important terpenoids while dehydroisoandrosterone acetate is a steroid. Borneol has been reported as an analgesia (Renee E. Granger et al., 2005), antihypertensive and antioxidant (Murugesan S.K. et al., 2010), anticoagulant and as antithrombotic compound (Li Y.H. et al., 2008). Camphene exhibits hypolipidemic (Ioanna Vallianou et al., 2011) and antinociceptive properties (Quintans-Junior L et al., 2013). β -

Eudesmol has been reported to exhibit antibacterial, anti-anoxic, antimutagenic and hepatoprotective properties, while caryophyllene has been reported as an anticancer, anti-inflammatory, anaesthetic, antimutagenic, antiseptic, antiparasitic and antimalarial compound (Cheryll Williams, 2011). α -Pinene is an important monoterpene exhibits antimicrobial (Ana Cristina Rivas da Silva et al., 2012), anti-inflammatory and anticatabolic (Ana T. Rufino et al., 2014), and anticancer properties (Beatrice Mercier et al., 2009). Ethyl cinnamate has been reported to exhibit nematocidal (In-Ho Choi et al., 2006) and larvicidal (K.V. Peter, 2006) properties, while ethyl-p-methoxycinnamate has been reported to exhibit larvicidal (Ahn,Y.-J. et al., 2008; Kim NJ et al., 2008), anti-carcinogenic (Xue Y., & Chen H., 2002), anti-tuberculosis (Lakshmanan D. et al., 2011) and anti-inflammatory (M.I. Umar et al., 2012) properties.

CONCLUSION

The essential oil of fresh rhizomes of *Kaempferia rotunda* L. was isolated by water distillation and analyzed by GC/MS method. Fifty seven compounds were identified, of which the major constituents were characterized as endo-borneol (9.30%), dehydroisoandrosterone acetate (9.12%),

naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl), [4aS-(4 α ,5 β ,8 α)]-(9.03%), and β -phellandrene

(7.08%). The essential oil yield and the percentage of identified compounds were 0.034 % and 99.74% respectively.

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