



CHEMICAL COMPOSITION OF ESSENTIAL OIL ISOLATED FROM THE RHIZOMES OF *KAEMPFERIA GALANGA L.*

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ABSTRACT

The aim of the present study was to evaluate the chemical composition of essential oil isolated from the rhizomes of *Kaempferia galanga L.* The essential oil was isolated from the dried rhizomes of *Kaempferia galanga L.* by steam distillation method and analyzed by gas chromatography-mass spectrometry (GC-MS) technique. Total fifty constituents were identified from rhizome oil constituting 97.19% of the oil. The major constituents of the oil were ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%), γ -cadinene (9.81%), 1, 8-cineole (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-m-methoxycinnamate (2.15%), camphene (1.58%), linoleoyl chloride (1.35%) and α -pinene (1.32%). The results suggest that the essential oil and its major components possess several biological activities like antibacterial, antifungal, analgesic, anti-inflammatory, antioxidant, antiviral, antihypertensive, anticarcinogenic, antinociceptive, anti-tuberculosis and larvicidal. The oil may be useful in perfumery, pharmaceuticals, and in aromatherapy as inhalants and massage to reduce anxiety, stress and depression.

KEY WORDS: *Kaempferia galanga L.*, Essential oil, GC-MS, Ethyl cinnamate



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INTRODUCTION

Plant essential oils and their constituents are known to have several biological activities^{1,2}. These biological activities include especially antimicrobial, antihelminthic, antiviral, anticancer, anti-inflammatory, antioxidant and insecticidal^{3,4}. Essential oils are composed by highly volatile and aromatic compounds of flavor and fragrances⁵. They are highly viscous organic liquids which are extracted through steam distillation or hydrodistillation from plant materials such as leaves, stems, roots, bark, flowers, fruits and seeds⁶. *Kaempferia galanga L.*, is an important oil yielding perennial herb, widely cultivated in India, Sri Lanka, Africa, and SE Asia particularly in Thailand and Malaysia⁷. *Kaempferia galanga L.*, commonly known as aromatic ginger, sand ginger (English), Sugandha-vachaa, Chandramula (Hindi), Karchura, or Shathi (Sanskrit), is an endangered Indian medicinal plant of *Zingiberaceae* family (PA Batugal et al., 2004). The rhizomes of this plant, are highly aromatic, have been used in several Asian countries as spice, food flavoring and folk medicines. Traditionally, the rhizomes have also been used to treat several ailments like coughs, furuncles, headache, diabetes, sore-throat, boils, parasite, rheumatism pain, amebiasis, parturition, flatulence, indigestion,

and tumor (James A. Duke, 2003). In India, the rhizomes of *Kaempferia galanga L.* have been used in Ayurvedic medicines for their stimulant, carminative, expectorant, and diuretic actions (C.P. Khare, 2007). Dried rhizomes of *Kaempferia galanga L.* contain 2.5 to 4% essential oil which is extensively used in food flavoring, perfumery, and in pharmaceuticals (K.V. Peter, 2004). Recent investigations revealed the potential antifungal, antibacterial, antibiofilm, antioxidant and antitumour activities^{8,9,10} of essential oil isolated from the rhizomes of *Kaempferia galanga L.*

EXPERIMENTAL PROCEDURE

Collection of plant material

Fresh rhizomes of the plant were collected from Sushila Tiwari herbal garden, Rishikesh, Uttarakhand, India in June, 2012. They were identified and authenticated by the staff of Botanical Survey of India, Dehradun, India and a voucher specimen (Acc. No. 114816) was deposited at the herbarium of Botanical Survey of India, Dehradun, India. Fresh rhizomes of the plant were washed in tap water and dried in shade for 30 days at room temperature. The plant is shown in *Figure 1*.



Figure 1
Kaempferia galanga L.

Isolation of essential oil

100g dried rhizomes of *Kaempferia galanga L.* were steam distilled for 8 hrs by using a Clevenger-type apparatus (Clevenger, 1928). The oil was extracted from the distillate with diethyl ether and then dried over anhydrous sodium sulfate. Thereafter, the solvent was evaporated to give yellowish essential oil which was subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of isolated oil was performed on a Shimadzu QP-2010 gas chromatograph coupled to a mass spectrometer (GC-MS) instrument (Shimadzu Corporation, Japan). The GC-MS was equipped with an autosampler.

Column: Rxi-5 Sil MS non polar fused silica capillary column (30 m length x 0.25mm diameter x 0.10 μm thickness, cross bond selectively, composed of 5% diphenyl and 95% dimethyl polysiloxate) was employed for the following conditions:

GC Oven Temperature program: 50 °C hold for 5 min. to 280 °C at the rate of 4 °C/min. and hold it for 10 min.

Carrier gas (99.999% purity)= helium @ 0.7 ml/min.

Injection volume= 1.5 μL of diluted oil in diethyl ether

Injector temperature= 250 °C

MS interface line temperature= 300 °C

Ion source temperature= 200 °C

Ionization energy= 70 eV

MS ion source= Electron impact ionization

Split injection ratio of 1:20

Scan mass range of m/z 40-600 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 08, FFNSC 1.2 and WILEY 8-Mass Spectral library of the GC-MS data software system.

galanga L. rhizomes essential oil by gas chromatography-mass spectrometry method which were significantly more than the number reported in literature^{11,12,13}. The retention data and chemical composition of essential oil are presented in *Table 1*. The major components of the oil were ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%), γ -cadinene (9.81%), 1,8-cineole (eucalyptol) (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-m-methoxycinnamate (2.15%), camphene (1.58%), linoleoyl chloride (1.35%) and α -pinene (1.32%).

RESULTS AND DISCUSSION

The steam distillation of the *Kaempferia galanga L.* rhizomes gave yellow color oil with a yield of 3.01%. Total fifty significant compounds were identified in *Kaempferia*

Table 1
The retention data and chemical composition of essential oil of *Kaempferia galanga L.* rhizomes using gas chromatography-mass spectrometry

RT	% Area	Compound
2.110	0.25	Ethyl acetate
2.226	0.77	sec.-Butyl ethyl ether
2.289	0.19	Benzene, Hexahydro-
2.330	0.04	iso-Butyrate, Isoamyl-
2.448	0.06	Ethyl-n-propyl ketone
2.514	0.21	Acetoin
2.631	0.24	Cyclohexane, Methyl-
2.882	0.08	Amyl acetate
2.980	0.09	Benzyl salicylate
3.022	0.28	Pentane, 3-Ethyl-3-methyl-
3.265	0.15	2-sec.-Butoxybutane
3.333	0.06	Heptan-2-ol
6.520	1.32	α -Pinene
7.062	1.58	Camphene
8.079	0.83	(-) β -Pinene
8.707	0.40	β -Myrcene
9.376	6.19	δ -Carene

10.004	0.74	p-Cymene
10.275	6.54	1, 8-Cineole
13.241	0.31	Dispiro [2.1.2.4] undecane, 8-Methylene
15.573	0.44	2,4-Cyclohexadiene-1-methanol, α , α , 4-Trimethyl-
15.892	5.21	Borneol
16.220	0.60	p-Menth-1-en-4-ol
16.334	0.74	Benzenemethanol, α , α , 4-Trimethyl-
16.548	0.71	p-Cymen-8-ol
16.825	0.65	α -Terpineol
17.218	0.15	Berbenone
17.327	0.66	Eucaevone
18.724	0.28	Verbenon
19.027	0.24	Benzaldehyde, p-Methoxy-
20.922	0.30	2-Pinen-4-one
21.588	0.37	Methyl geranate
23.821	0.35	β -Elemene
24.147	0.69	Azulene
24.381	0.53	Octadecyl chloride
24.720	0.24	Caryophyllene
25.897	0.26	α -Humulene
26.745	29.48	Ethyl cinnamate
27.053	0.20	β - Selinene
27.872	9.81	γ -Cadinene
28.069	0.66	δ - Cadinene
28.966	0.34	α -Elemol
29.193	0.27	γ -Elemene
32.053	0.73	Hedycaryol
32.311	2.15	Ethyl-m-methoxycinnamate
32.693	0.22	Cyclododecene, (E)-
32.944	1.35	Linoleoyl chloride
33.662	0.44	n-Hetadecane
35.288	18.42	Ethyl-p-methoxycinnamate
40.358	0.37	Epimanool
97.19		Total Identified Constituents

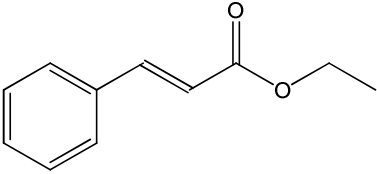
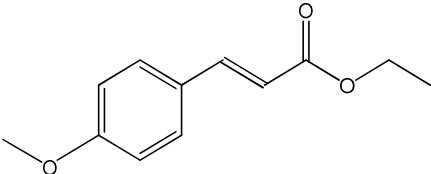
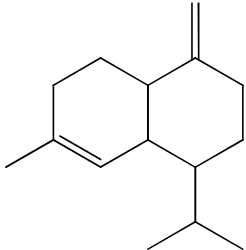
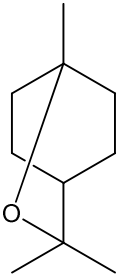
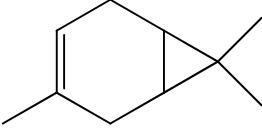
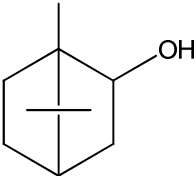
RT= Retention Time on Rxi-5 Sil MS column; identification was done by NIST 08, FFNSC 1.2 and WILEY 8-MS Spectral library of the GC-MS data software system.

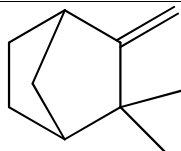
Biological activities of major constituents

Gas chromatography-mass spectrometry analysis showed that the essential oil from the rhizomes of *Kaempferia galanga L.* is a complex mixture of volatile monoterpenes, oxygenated terpenes and sesquiterpenes as well as non-terpene hydrocarbons, esters, fatty acids and ketones. Ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%), γ -cadinene (9.81%), 1,8-cineole (eucalyptol) (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-m-methoxycinnamate (2.15%), camphene (1.58%), linoleoyl chloride (1.35%) and α -pinene (1.32%) were found as the major constituents of the rhizome oil. These compounds have been reported to exhibit significant biological activities. These biological activities together with the structure of major constituents are summarized in Table 2. Ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%) and ethyl-m-methoxycinnamate (2.15%) are esters which contribute the nematocidal, anticancer,

antituberculosis, anti-inflammatory, antifungal and larvicidal properties to the oil. γ -cadinene (9.81%), 1, 8-cineole (6.54%), camphene (1.58%) and α -pinene (1.32%) are monoterpenes while borneol (5.21%) and δ -carene (6.19%) are oxygenated terpene and sesquiterpene respectively. Monoterpenes and sesquiterpenes were found in the essential oil of *Kaempferia galanga L.* rhizomes which also may contribute the flavor and fragrance properties to the oil. They have a wide range of biological activities mentioned in Table 2. Linoleoyl chloride is a fatty acid used in the synthesis of a large number of organic polymers^{14,15,16}. Essential oils are known as novel human skin penetration enhancer for transdermal drug delivery¹⁷ and also have antihypertensive potential. Therefore, the isolated oil may be useful in aromatherapy; the fragrances of its volatile molecules help to reduce anxiety, stress, tension and inflammation of the patients.

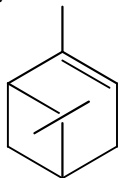
Table 2
The biological activities of major constituents with their chemical structure

Key constituents with their structures	Biological activities
Ethyl cinnamate (29.48%) 	i) Larvicidal activity (K.V. Peter, 2006) ii) Anticarcinogenic activity (Xue, Y., Chen H., 2002) iii) Nematicidal activity (In-Ho Choi et al., 2006)
Ethyl-p-methoxycinnamate (18.42%) 	i) Larvicidal activity (Ahn, Y.-J. et al., 2008; Kim NJ et al., 2008) ii) Antiangiogenic effects (Zhi-Heng He, 2012) iii) Anti-tuberculosis activity (Lakshmanan D. et al., 2011) iv) Antifungal activity (Gupta S.K. et al., 1976) v) Anticancer activity (Xue Y. and Chen H., 2002) vi) Anti-inflammatory activity (M.I. Umar et al., 2012)
γ-Cadinene (9.81%) 	i) Fungal metabolite (David E. Cane et al., 1987) ii) Aroma compound (http://www.uniprot.org/uniprot/B2KSJ5)
1, 8-Cineole (6.54%) 	i) Insect repellent activity (Obeng-O fori D. et al., 1997) ii) Analgesic activity (Masayuki Takaishi et al., 2012) iii) Anti-inflammatory and Antinociceptive activities (Santos, FA and Rao VS, 2000; Juerqens UR et al., 2003) iv) Antibacterial and cytotoxic activities (Afroditi Sivropoulou et al., 1997) v) Antioxidant potential and lipid-lowering effect (Kyung-Hyun Cho, 2012) vi) Antiexudant, cytotoxic, analgesic and antitumor activities (Zh. K. Asanova et al., 2003) vii) vasorelaxant activity (S. Lahlou et al., 2002) viii) Fumigant activity (V. Rozman et al.,)
δ-Carene (6.19%) 	i) Antibacterial and expectorant potential (Niccolini P. et al., 1964) ii) Anti-inflammatory, antiseptic and dermatitogenic activities (James A. Duke, 2003)
Borneol (5.21%) 	i) Antihypertensive and antioxidant potential (Murugesan SK et al., 2010) ii) Anticoagulant and antithrombotic activities (Yan-Hong Li et al., 2008) iii) Analgesic potential (Renee E. Granger et al., 2005)
Camphene (1.58%)	i) Insecticide potential (D. Prasad, 2007) ii) Antinociceptive activity (Quintans-Junior L et



al., 2013)
 iii) Hypolipidemic activity (Ioanna Vallianou et al., 2011)

α -Pinene (1.32%)



i) Antimicrobial potential (Ana Cristina Rivas da Silva et al., 2012)
 ii) Anticancer activity (Beatrice Mercier et al., 2009)
 iii) Antinociceptive activity (Aydin Him et al., 2008)
 iv) Antiviral activity (Astani A. et al., 2010)
 v) Insecticide potential (D Prasad, 2007)

CONCLUSION

The essential oil of *Kaempferia galanga L.* was isolated from its rhizomes by steam distillation and analyzed by gas chromatography-mass spectrometry technique. Fifty constituents were identified representing 97.19% of the oil and major constituents were ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%), γ -cadinene (9.81%), 1,8-cineole (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-methoxycinnamate (2.15%), camphene

(1.58%), linoleoyl chloride (1.35%) and α -pinene (1.32%). Esters and terpenoid compounds were the major constituents of essential oil of *Kaempferia galanga L.* rhizomes. The study of the biological activities of major constituents of *Kaempferia galanga L.* essential oil suggests that the oil exhibits a wide array of biological activities and may be used in aromatherapy, cosmetics, food flavoring, fragrances and pharmaceuticals.

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