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Note

Chemical composition and antimicrobial activity of the essential oil of *Alpinia officinarum* rhizome

A K Indrayan*[a], S N Garg[b], A K Rathi[a] & V Sharma[c]

[a]Natural Products Laboratory, Department of Chemistry, Gurukula Kangri University, Hardwar 249 404, India

[b]Medicinal Plant Chemistry Division, Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India

[c]Present address : Research Scientist, Chembiotek Research International, Salt Lake Electronic Complex, Kolkata 700 091, India

E-mail: akindray@sancharnet.in

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The essential oil of the rhizome of *Alpinia officinarum* collected from West Bengal, India has been isolated by hydrodistillation and analysed by means of GLC and GC-MS in apolar and polar columns. Forty-nine compounds representing 99.21% of the oil have been identified. The major constituents of the essential oil of the rhizome are 1,8-cineole (55.39%), Δ^3-carene (8.96%), β-pinene (4.29%), camphene (2.81%), limonene (2.80%), *iso*caryophyllene (2.52%), camphor (2.35%), α-pinene (2.27%), γ-terpinene (2.23%) and γ-cadinene (2.17%). The oil shows antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive), *Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi* (Gram negative), and antifungal activity against *Candida albicans.*

Keywords: *Alpinia officinarum*, lesser galangal, Chinese ginger, Zingiberaceae, essential oil, composition, 1,8-cineole, antimicrobial activity

Alpinia officinarum Hance (Zingiberaceae), also known as 'lesser galangal', 'Chinese ginger', 'China root', 'radix galangae minoris' or 'small galangal' is a plant that grows in India and South Eastern China[1]. In India it is cultivated in the plains of West Bengal, Assam and in the Eastern Himalayas[2]. Botanically related to true ginger (*Zingiber officinale* Roscoe), the reed–like plant attains a height of about 1 m (Ref. 1). It is a perennial herb with thick, creeping, reddish brown rhizomes, narrowly lanceolate-acuminate, ornamental leaves, and racemes of showy white flowers[2]. The rhizome has a peculiar eucalyptus-cardamom-ginger type odour and the taste is hot and spicy. It has been used in both Ayurvedic and Chinese medicine against a variety of diseases[3], for improving sexual potency, in treatment of jaundice[4] and for antidermatophytic activity[5]. Some components are COX-2 inhibitors6. The first study of lesser galangal oil was reported by Lawrence *et al.*[7] from a Chinese sample. Authors used chromatographic techniques in combination with IR spectroscopy and in a very good effort could identify many of the components but not all of them. Zhao *et al.*[8] compared the chemical composition of the volatile oil of wild Chinese *Alpinia officinarum* with that of cultivated. A study of the essential oil from the rhizomes of the Vietnamese sample by GC and GC/MS was reported by Leclercq *et al.*[9] but 1,8-cineole was not present among the main components. More recently, Tram *et al.*[10] in a similar study could isolate many more compounds along with 1,8-cineole as the main constituent (50.0%) from the fresh rhizome. Only one antimicrobial study on the essential oil of the rhizome is available in literature and that too only on the antifungal properties. Ray *et al.*[11] determined the antifungal action against *Candida albicans* and a few other fungi. Recently, however, Moo *et al.*[12] have reported growth inhibitory effect of this essential oil against certain human intestinal bacteria.

The present study describes the isolation of essential oil and the analysis of the isolated oil by GC and GC/MS alongwith antimicrobial evaluation against two Gram positive and three Gram negative bacteria and one fungus.

Results and Discussion

The yield of essential oil obtained from Rhizomes of *Alpinia officinarum* was 0.9% w/w. Constituents were identified representing 99.21% of the oil. They are summarised in **Table I**.

As can be seen from **Table I**, 99.21% of the constituents have been identified. 25 of the constituents are monoterpene hydrocarbons and derivatives representing 85.99% of the total essential oil including 12 oxygenated monoterpenes. The major monoterpenes and derivatives are 1,8-cineole (55.39%), Δ^3-carene (8.96%) and β-pinene (4.29%). Monoterpenes possess analgesic, antiseptic, expectorant and stimulating properties. Some are antiviral and some help break down gall stones. It should be noted that the corresponding proportion of

Table I — Constituents of the essential oil from rhizome of *Alpinia officinarum*

Compd	Retention time (min)		Percent	Identification
	Apolar	Polar		
α-Thujene	6.005	1.14	0.29	RT
α-Pinene	6.848	1.41	2.27	RT, GC/MS
Camphene	7.287	2.02	2.81	RT, GC/MS
*iso*Butyl isobutyrate	7.786	2.14	0.58	RT
β-Pinene	7.998	2.26	4.29	RT, GC/MS
β-Myrcene	8.182	3.07	0.31	RT
l-Phellandrene	8.400		0.09	RT
α-Terpinene	8.528		0.13	RT
Unidentified	8.941		0.11	
trans-Ocimene	9.167		0.43	RT
Limonene	9.331	3.42	2.80	RT
1,8-Cineole	9.540	4.04	55.39	RT, GC/MS
p-Cymene	10.215	4.32	0.25	RT
Unidentified	11.284		0.24	
Citronellol	11.392	5.01	0.47	RT
α-Terpinolene	12.374	5.17	0.14	RT
β-Santalene	13.329		0.20	RT
Camphor	13.700	10.52	2.35	RT, GC/MS
γ-Elemene	13.889		0.20	RT
trans-β-Farnesene	14.331		0.42	RT
δ-Terpineol	14.482	12.28	0.37	RT
α-Humulene	14.622	13.15	0.14	RT
γ-Terpinene	14.806	13.32	2.23	RT, GC/MS
Δ^3-Carene	15.296	16.12	8.96	RT, GC/MS
Fenchyl acetate	16.433		0.65	RT, GC/MS
Benzyl acetone	17.381		0.35	RT, GC/MS
Linalool	19.243		0.16	RT
α-Santalene	20.938		0.18	RT
trans-Pinocarveol	23.188		0.20	RT
*endo*Borneol	23.785		0.20	RT
Bornyl acetate	25.109		0.26	RT
Caryophyllene	25.482		1.28	RT, GC/MS
Bergamotene	25.693		1.13	RT, GC/MS
β-Elemene	25.993		0.18	RT
p-Cymen-8-ol	26.597		0.24	RT
Terpinen-4-ol	26.949		0.43	RT
β-Sesquiphellandrene	27.671		0.25	RT
γ-Muurolene	27.810		0.17	RT
*iso*Caryophyllene	28.353		2.52	GC/MS
Calamenene	28.548		0.17	RT
α-Farnesene	28.684		0.63	RT, GC/MS
α-Selinene	28.979		0.19	RT, GC/MS

— *Contd*

Table I — Constituents of the essential oil from rhizome of *Alpinia officinarum* — *Contd*

Compd	Retention time (min)		Percent	Identification
	Apolar	Polar		
γ-Cadinene	29.337		2.17	RT, GC/MS
δ-Cadinene	29.565		0.30	RT, GC/MS
Selina-4,11-diene	30.378		0.23	RT, GC/MS
Calacorene	30.677		0.44	RT, GC/MS
Selina-3,7(11)-diene	32.551		0.43	RT
*iso*Ledene	33.637		0.34	RT, GC/MS
Unidentified	34.366		0.13	
Butyl benzoate	35.182		0.24	RT
Phenyl ethyl-2-methyl butyrate	35.387		0.39	RT
Unidentified	35.682		0.10	
Unidentified	36.768		0.10	
Unidentified	37.066		0.14	
Caryophyllene oxide	37.241		0.36	RT

Table II — Antimicrobial activity of essential oil from rhizome of *Alpinia officinarum* compared with a standard

Microorganism	Inhibition zone (mm)	
	Essential oil	Standard (1%)
Bacteria		
Staphylococcus aureus (+)	17.1	30.0
Bacillus subtilis (+)	12.3	25.1
Escherichia coli (–)	15.0	21.8
Klebsiella pneumoniae (–)	12.5	25.0
Salmonella typhi (–)	14.7	20.5
Fungus		
Candida albicans	18.0	28.3

Standard was streptomycin for antibacterial and griseofulvin for antifungal study

the monoterpene hydrocarbons and derivatives 1,8-cineole, Δ^3-carene and β-pinene is higher in the Indian than the Vietnamese sample[10]. No Δ^3-carene (8.96% in the present sample) has been found in Chinese and Vietnamese samples. Also, in the Indian sample the percentage of 1,8-cineole is higher. 1,8-cineole serves as a stimulating expectorant in cases of chronic bronchitis. It also finds application in room sprays, lotions, and in many varieties of cosmetic preparations. In the present studied sample, the total percentage of oxygenated constituents is 62.02%. Presence of l-phellandrene, *trans*-ocimene, citronellol, Δ3-carene, *trans*-pinocarveol, *endo*borneol and *p*-cymen-8-ol are reported here for the first time in *Alpinia officinarum* oil sample.

Sesquiterpenes and derivatives constitute 12.58% of the oil in the present sample. *Iso*caryophyllene (2.52%) and γ-cadinene (2.17%) being the major components. Presence of the sesquiterpenes β-sesquiphellandrene, *iso*caryophyllene and *iso*ledene are reported here for the first time in the *A. officinarum* oil. Sesquiterpenes have antiinflammatory properties and work as liver and gland stimulants. They have been found more recently to reach across the blood brain barrier, increasing oxygen around the pineal and pituitary glands. Previous studies have shown that the essential oils and components thereof hold promise as antimicrobial agents for various uses in medical application[3]. In the present study, the essential oil of the *Alpinia officinarum* was tested for its antimicrobial properties against human pathogenic bacteria. It showed good inhibitory activity against the Gram (+) human pathogens *Staphylococcus aureus* , *Bacillus subtilis* and Gram (–) *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and antifungal activity against *Candida albicans* (**Table II**).

The study indicates the Indian *A. officinarum* oil to be somewhat different from the Chinese and Vietnamese oils.

Plant Materials

The reddish brown rhizomes were collected form Kolkata, West Bengal (India) in December 2003 and duly identified through Forest Research Institute (ICFRE) Dehradun. A voucher specimen has been deposited in the Herbarium of the Plant Medicine Section of the Chemistry Department of Gurukula Kangri University under the registry no. 12/15. The rhizomes were washed with luke warm water and air dried.

Experimental Section

Isolation of Essential Oil

The oil was obtained by hydrodistillation for 3 hr using a Clevenger apparatus. The lighter than water transparent oil was re-extracted with diethyl ether and dried over anhydrous Na_2SO_4. The light yellow transparent oil having spicy odour resembling that of cardamom and myrtle and a faintly bitter taste with a cooling after effect was obtained with yield 0.9% w/w, d^{25} 0.922 g mL−1; $[\alpha]_{d}^{18}$ +9°45′; n^{18} 1.4550; acid number 4.49; ester number 22.44; acetyl value 43.74; ester number after acetylation 65.45; iodine value 65.40; clearly soluble in ethanol, carbon tetrachloride, diethyl ether and chloroform.

Gas Chromatography

The essential oil sample was subjected to GC analysis using both polar and apolar columns. Varian Gas Chromatograph CX-3000 was equipped with the apolar PE-5 column (equivalent DB-5) (50 m × 0.32 mm; film thickness 0.25 µm for less polar and 0.5 µm for polar medium). Oven temperature was kept at 100°C for 2 min and programmed to 230°C at a rate of 3°C/min and kept constant at 230°C for 4 min; carrier gas: hydrogen; injection port temperature: 220°C; detector (FID) temperature: 250°C. Polar column was of similar dimensions DB Wax (equivalent to OV-1/SE-30). The percentage compositions were computed by taking the help of GC peak areas.

Gas Chromatography/Mass Spectrometry

GC–MS analysis of the essential oil was carried out on a Perkin Elmer Turbo mass instrument with Auto XLGC equipped with a PE-5 column (50 m × 0.3 mm; film thickness was 0.25 µm for apolar and 0.5 µm for the otherwise similar sized polar column and interfaced with ion source detector). Chromatographic conditions were as follows — carrier gas: helium (5 psi to 10 psi @ 1 psi/min); injector temperature: 220ºC; detector temperature 280ºC; oven temperature: 100ºC to 250°C @ 3°C/min. The column was coupled directly to the quadrapole mass spectrometer operated in the electron ionisation (EI) mode at 70 eV. The components were identified by comparing the mass spectra and retention times (R*t*). Library search was carried out using Wiley, NBS and NIST GC/MS Library. Peak areas helped in determining the percentage composition.

Antimicrobial Studies

A collection of 6 microorganisms was used, including the Gram (+) bacteria *Staphylococcus aureus* (MTCC 737) and *Bacillus subtilis* (MTCC 1789), Gram (–) *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 2405), and *Salmonella typhi* (MTCC 53) and the fungus *Candida albicans* (MTCC 227). The test pathogenic bacteria were maintained on nutrient agar medium (NAM) slants at 4°C. The paper disc diffusion plate method was used to determine inhibitory zone of pure essential oil and the streptomycin standard (1% aqueous solution) for antibacterial studies.

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