Comparative chemical study of two varieties of attractive medicinal plant *Kaempferia galanga* Linn.

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Abstract

'Kasthuri' and 'Rajani' varieties of medicinal and ornamental plant ***Kaempferia galanga* Linn.** differ morphologically. The essential oils from their rhizomes have remarkably different specific gravities, refractive indices, saponification and iodine values. These oils also differ sufficiently in their chemical compositions. A total no. of 58 and 56 compounds have been identified in 'Kasthuri' and 'Rajani', respectively. Thirteen compounds are identified in the rhizome oil of 'Kasthuri' that are not present in the rhizome oil of 'Rajani' and another 11 compounds identified in 'Rajani' oil are not present in 'Kasthuri' oil. Forty-five compounds have been found common in both oils but their percentages differ in the two varieties. Similarly major component, ethyl-trans- *p*-methoxycinnamate is present in both the varieties, its percentage varies (39 and 35%, respectively).

Keywords : *Kaempferia galanga,* Zingiberaceae, Essential oil composition, 'Kasthuri', 'Rajani', Ethyl-trans-*p*-methoxycinnamate.

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Introduction

***Kaempferia* Linn.** (Family: Zingiberaceae) is a genus of about 70 species of rhizomatous, aromatic perennial herbs, found in Africa and South East Asia. A few species are grown as ground cover in the tropics or under cover in cold areas. They need high humidity, and do well in pots alongside warm-growing orchids or in beds beneath green house staging[1]. ***Kaempferia galanga* Linn.**, known as *Chandramula* in Hindi and *Kacholam* in Malayalam, is an attractive plant used in medicines and for variety of other purposes. It is cultivated mainly in India, China, Malaysia and Indonesia. Leaves two or three, spreading flat on the ground, rotund-ovate or suborbicular, deltoid-acuminate, thin, green; flowers white, 6-12 on a short scape, fugacious, fragrant opening successively: lip bilobed with lilac or purple spots[2,3]. The tuberous rhizomes possess a camphoraceous odour with somewhat bitter aromatic taste.

Fertile loamy soil having good drainage is ideal for the crop. Laterite soil with heavy organic manure application is also well suited. Land is prepared to a good tilth during summer by ploughing or digging. On receipt of pre-monsoon

Rhizome var. Kasthuri — Rhizome var. Rajani

Kaempferia galanga

showers, beds of 1 m width 25 cm height are prepared with spacing of 40 cm between beds[4]. Whole or split rhizome with at least one healthy sprout is the planting material. FYM or compost is applied as basal dose at the rate of 20 tonnes/ha by broadcasting and ploughing. N, P_2O_5 and K_2O at the rate 50, 50 and 50 kg/ha, respectively are applied at the time of the first and second weeding. Gamma radiation doses between 0.5-1.0 kR resulted in 100% sprouting[5]. Maheswarappa *et al* have studied the influence of planting material, plant population and organic manures on the plants grown as intercrop in coconut garden[6].

The powder of tubers mixed with honey is given in coughs and pectoral affections[3]. The rhizomes are considered stimulating, expectorant, carminative and diuretic[2]. In Philippines, a decoction of the rhizomes is used for dyspepsia, headache and malaria[2]. Roasted rhizomes are applied hot in rheumatism and for hastening the ripening of inflammatory tumours[2]. They are also used as a wash in dandruff and for relieving irritation produced by stinging caterpillars[2]. The leaves are used in lotions and poultices for sore eyes, sore throat, swellings, rheumatism and fevers[2]. A Japanese study has reported the presence of anticancer principles in the rhizome[7]. Recently the vasorelaxant effect of compound isolated from the plant on smooth muscles of the rat aorta has been reported[8]. Achuthan and Padekkala have reported the hypolipidemic action of the ethanolic extract of the plant *in vivo*[9]. The amoebicidal[10] and insecticidal[11] activities have been reported in Indonesian sample. Larvicidal principles have also been obtained from the methanol extract of the rhizome[12]. Anti-carcinogenic effect of three compounds isolated from this plant has been reported by Xue and Chen[13]. An ethanolic extract of an Indonesian plant sample has shown some antiplatelet aggregating activity[14]. Recently the Thai species of the plant has been screened for antioxidant activity[15].

In India and many other parts of Asia, the rhizomes and leaves of the plant are employed as a perfume in cosmetics, hair washes and face powders and sometimes worn by women for fragrance[2]. They are chewed with betel nut[2]. They are also used to protect the clothing from insects[2]. In Malaysia the rhizomes are used for flavouring of curries[16] and also for chills in elephants[2]. The use of compounds from the rhizome in sunscreen formulations has also been reported[17].

Some researchers have reported the chemical composition of the essential oil from the rhizome. Ding *et al*[18] reported 30 constituents and Qui *et al*[19] reported 34 constituents in samples from China. While, 53 compounds were reported by Wong *et al*[20] in a Malaysian sample, Sudibyo[21] could identify only eight components in the oil from Indonesia and Tewtrakul *et al*[22] reported nine compounds in a Thailand sample.

An interesting observation came to light when in the year 2002 the scientists from Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara (Kerala) released two varieties of ***K. galanga***, 'Rajani' and 'Kasthuri'[23] as per TA (2) 38059/02 dt 07.10.2002 of Director of Agriculture, Kerala. They released these two varieties in an effort to increase the yield of the highly useful rhizome. They claimed these two varieties to be with an yield potential of more than 2 tonnes dry rhizomes per ha and altogether having a good aroma and flavour. The two varieties differed in colour of rhizome- light brown in 'Kasthuri' and creamish white in 'Rajani'. To investigate further differences and the chemical compositions of the two rhizome oils, present study was done on cultivation as well as chemical composition.

Materials and Methods

Cultivation

Authenticated fresh rhizome of the two varieties 'Kasthuri' and 'Rajani' were procured from Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur (Kerala) and samples deposited in the Herbarium of Plant Medicine Section of the Chemistry Department of Gurukula Kangri University, Hardwar, Uttarakhand. Fresh rhizomes were planted in differently marked pots having the same soil and kept under identical conditions. Equal watering was done time to time as and when required in the grown plants.

A morphological study of the grown plants of the two varieties was also carried out.

Isolation of rhizome oil

The rhizomes from the two varieties were collected separately, washed with luke warm water and dried in shade. They were grounded to coarse powder. Isolation of essential oil was carried out separately by hydrodistillation using

Clevenger apparatus. After the complete distillation, the oil was permitted to stand undisturbed and extracted with anhydrous sodium sulphate to remove any aqueous part. It was stored in a dark coloured air-tight bottles under refrigeration. The percentage yields in both the cases were noted.

Physico-chemical properties of essential oils

For both the oils the physical appearances (physical state, colour and odour), specific gravity, refractive index, optical rotation, acid value, saponification value and iodine value were determined by the methods described by Garratt[24], Guenther[25] and in monographs of I.S.I[26].

Chemical composition of essential oils

Both the oil samples were subjected to GC/MS analysis to investigate the difference in their chemical composition.

Gas chromatography: The oil samples were subjected to GC analysis. Perkin Elmer Gas Chromatograph Clarus 500 equipped with DB-5MS (60 m × 0.32 mm; film thickness 5µm) was used. Oven temperature was kept 65ºC for 4 minutes and programmed to 250ºC at a rate of 4ºC/min and kept constant at 250ºC for 20 minutes. Carrier gas: nitrogen; flow rate: 1.5 mL/min; split ratio: 1:10; injection temperature: 240ºC, detectors temperature: 250ºC. The percentage compositions were computed electronically from the GC peak areas without using any correction factors.

Gas chromatography/Mass spectro-metry: GC/MS analysis of the oils was carried out on Shimadzu GC-2010, GCMS-QP 2010 equipped with similar column and operated under similar conditions as GC. Carrier gas: helium; flow rate: 24 cm/s (linear velocity); split ratio: 1:5; detector voltage: 1 KV; ion source temperature: 200ºC. The components were identified by comparing mass spectra and retention times. Library search was carried out using NIST 12 and NIST 62 GC/MS Libraries. Peak areas helped in determining the percentage composition.

Results and Discussion

The cultivation of procured fresh rhizomes gave successful results. Plants of the two varieties grew gradually and simultaneously. The observed morphological characters of the two varieties of plant are given in Table 1. The demarkable morphological character of both the varieties is the colour of rhizomes. The percentage yields of the essential oils obtained from the two varieties and the physico-chemical properties of the two oils are summarized in Table 2.

Table 1 : Morphological characters of the two varieties of *Kaempferia galanga*

Character	'Kasthuri'	'Rajani'
Colour of rhizome	Light brown	Creamish white
Health of plant	Robust (shining, thicker leaves)	Less healthy (less shining, comparatively thinner leaves)
Number of leaves in plant	More	Fewer
Size of leaves	Larger (10-15 cm)	Smaller (7.5-12.5 cm)

Table 2 : Percentage yield and physico-chemical characters of the essential oils from the two varieties of *Kaempferia galanga*

Yield / Property	'Kasthuri'	'Rajani'
Yield of essential oil	1.88%	1.76%
Physical state	Not a much viscous liquid	Not a much viscous liquid
Colour	Light yellow	Pale yellow
Odour	Camphoraceous	Camphoraceous
Specific gravity (30°/30°C)	0.9882	0.9950
Refractive index (30°C)	1.491	1.512
Specific rotation (30°C)	- 4°15'	- 4°10'
Acid value	2.10	2.52
Saponification value	92.2	102.3
Iodine value	98.8	108.4

Though the yields and colours of oils obtained from rhizomes of 'Kasthuri' and 'Rajani' differ slightly, there is difference in specific gravities and refractive indices; values of both are higher in the case of 'Rajani' oil (Table 2). This oil has larger unsaturation as indicated by its higher iodine value; saponification value is also higher.

The results (Table 3) indicate that in this comprehensive study not only many different compounds have been determined in the rhizome oil of ***K. galanga***, which were not reported hitherto, but also the two varieties differ remarkably. Whereas only 30 constituents were identified by Ding *et al*[18] and 34 by Qui *et al*[19] in Chinese samples,

Table 3 : Comparative chemical composition of rhizomes oil from 'Kasthuri' and 'Rajani'

Compounds	'Kasthuri'		'Rajani'		Retention index for both varieties
	Retention time (min.)	Per cent	Retention time (min.)	Per cent	
2-Heptanol	-	-	18.650	0.02	877
Styrene	18.850	0.01	-	-	893
α-Thujene	20.300	0.07	20.300	0.05	938
Tricyclene	20.450	0.11	20.450	0.08	941
α-Pinene	20.850	0.97	20.850	0.73	945
Camphene	21.800	1.98	21.800	1.67	953
α-Phellandrene	22.600	0.17	22.600	0.12	989
β -Myrcene	22.850	0.17	22.850	0.12	992
β -Pinene	23.050	0.39	23.050	0.38	994
Δ-Carene	24.250	7.86	24.250	5.58	1009
p-Cymene	24.550	0.06	24.550	0.03	1027
m-Cymene	24.850	1.05	24.850	0.71	1028
D-Limonene	25.050	0.90	25.100	0.71	1033
1,8-Cineole	25.400	8.07	25.400	13.57	1077
Z-3-Octen-1-ol	25.850	0.05	-	-	1072
1-Octanol	26.250	0.31	26.250	0.17	1073
Artemisia alcohol	-	-	27.200	0.04	1084
trans-Isomyrcenol	27.500	0.47	27.500	0.43	1090
β-Linalool	27.650	0.06	27.650	0.14	1098
(S)-cis-Verbenol	28.600	0.08	28.600	0.04	1138
cis-Limonene oxide	-	-	29.050	0.02	1142
2-(2-Methyl-1-propenyl)-1, 5-cyclohexadiene	29.450	0.28	29.450	0.10	1140
cis-2-(4-Methylcyclohexyl)-propan-2-ol	29.650	0.16	29.650	0.08	1144
Unidentified	30.300	0.38	30.300	0.21	
Camphor	30.550	0.11	30.550	4.25	1143
α-Phellandren-8-ol	30.750	0.92	30.750	0.34	1159
trans-Dihydro-α-terpineol	31.100	0.28	-	-	1160
Isoborneol	-	-	31.200	2.11	1161
Terpinen-4-ol	31.250	0.95	-	-	1172
Borneol	31.450	3.36	31.450	2.57	1168
p-Cymen-8-ol	31.600	0.71	31.600	0.60	1183
α-Terpineol	32.050	0.35	32.050	0.45	1189

Compounds	'Kasthuri'		'Rajani'		Retention index for both varieties
	Retention time (min.)	Per cent	Retention time (min.)	Per cent	
Eucarvone	32.900	1.35	32.900	0.69	1223
Carvone	-	-	34.000	0.06	1242
Perilla aldehyde	34.250	0.42	34.250	0.17	1261
p-Anisaldehyde	34.500	0.11	34.500	0.10	1262
Thymol	35.550	0.12	35.550	0.11	1290
Bornyl acetate	36.650	0.20	36.650	0.12	1302
α-Terpinyl acetate	37.550	0.05	37.550	0.03	1350
Ethyl-cis-cinnamate	37.950	0.08	37.950	0.04	1361
Carvone oxide	38.950	0.15	38.950	0.13	1363
β-Elemene	39.400	0.10	39.400	0.49	1391
α-Gurjunene	40.400	0.12	40.400	0.10	1409
β-Caryophyllene	-	-	40.500	0.27	1418
β-Humulene	40.750	0.11	40.750	0.08	1440
δ-Selinene	-	-	40.900	0.09	1472
Ethyl trans-cinnamate	41.850	18.83	41.800	13.14	1474
Germacrene D	43.000	0.07	43.000	0.21	1492
n-Pentadecane	42.250	3.22	42.250	2.41	1496
Eremophilene	-	-	43.250	0.34	1494
Germacrene A	-	-	43.400	0.10	1502
δ-Cadinene	43.750	0.58	43.750	0.50	1524
Unidentified	44.350	0.57	44.350	0.29	
Caryophyllene oxide	44.700	0.18	44.700	0.13	1574
(-)-Spathulenol	46.050	0.19	46.050	0.13	1576
Germacrene D-4-ol	-	-	46.350	7.97	1577
Dendrolasin	46.350	0.49	-	-	1578
Cedrol	47.050	0.27	-	-	1579
1-Hexadecene	47.200	0.25	-	-	1578
Hexylbenzoate	47.500	0.52	47.500	0.45	1580
Davanone	48.000	0.77	48.000	0.60	1581
Ledol	48.200	0.42	48.200	0.18	1583
(-)-Globulol	48.550	0.26	48.550	0.34	1588
α-Bisabolol	48.900	0.12	-	-	1683
Juniper camphor	-	-	49.800	0.33	1691
Heptadecane	49.800	0.27	-	-	1700
Ethyl- trans-*p*-methoxy cinnamate	50.750	39.09	50.700	35.09	1773
Pentadecanol	51.900	0.12	-	-	1866
Hexadecanol	53.250	0.09	-	-	1870
Unidentified	53.850	0.12	-	-	
Kaur-16-ene	59.650	0.27	-	-	2070
17-(Acetyloxy)-kauran-18-al	67.300	0.22	-	-	-

Wong *et al*[20] were successful in identifying 53 compounds in a Malaysian sample, 34 through OV-101 column and another 19 through Carbowax 20M column that included the compounds like indole and vanillin. In another study Jirovetz *et al*[27] reported more than 65 constituents in oil from South India, but present study has indicated many different results. In our study, out of the 58 compounds identified in 'Kasthuri' oil and 56 identified in 'Rajani' oil, 45 compounds are found common in both oils, but 13 compounds that are present in 'Kasthuri' oil are not present in 'Rajani' oil, while 11 compounds that are present in 'Rajani' oil are absent in 'Kasthuri' oil. Ethyl-trans-*p*-methoxycinnamate is the major constituent in both of them (39.09 % in 'Kasthuri', 35.09 % in 'Rajani') followed by ethyl-trans-cinnamate (18.83 and 13.14 %), 1,8-cineole (8.07 and 13.57%). Ethyl-trans-*p*-methoxycinnamate and ethyl-trans-cinnamate both show monoamine oxidase inhibiting and larvicidal effects[20, 28]. The cinnamate derivatives are responsible for the aromatic spicy odour impression also[27]. 1,8-Cineole serves as a stimulating expectorant in cases of chronic bronchitis. It is also used in room sprays, lotions, and in all kinds of cosmetic preparations. The compound Δ-carene is 7.86% in 'Kasthuri' whereas 5.58% in 'Rajani'; the germacrene D-4-ol is found in 'Rajani' (7.97%) only. Camphor is 4.25% in 'Rajani' but only 0.11% in 'Kasthuri'. Thirteen compounds styrene, Z-3-octen-1-ol, trans-dihydro-α-terpineol, terpinen-4-ol, dendrolasin, cedrol, 1-hexadecene, α-bisabolol, heptadecane, pentadecanol, hexadecanol, kaur-16-ene and 17-(acetyloxy)-kauran-18-al are found to be present in 'Kasthuri' but absent in 'Rajani', whereas another eleven compounds 2-heptanol, artemisia alcohol, *cis* limonene oxide, isoborneol, carvone, β-caryophyllene, δ-selinene, eremophilene, germacrene A, germacrene D-4-ol and juniper camphor are present in 'Rajani' but not in 'Kasthuri' oil.

The percentage of monoterpenes in 'Kasthuri' oil is 30.57 out of which 16.31% are the oxygenated whereas in 'Rajani' oil it is 35.92 out of which 25.64% are oxygenated. Monoterpenes are analgesic, antiseptic, expectorant and stimulating compound. Some are antiviral and some help break down gallstones. Monoterpene derivatives like 1,8-cineole, borneol, Δ-carene and carvone possess pleasant fresh-odour notes[27]. The quantity of sesquiterpenes is comparatively very low. The diterpenes are present in 'Kasthuri' oil only; they are only 0.49% including 0.22% oxygenated diterpene.

Presence of eremophilene in essential oils is not very common. Its presence has recently been reported in seed extract of ***Piper nigrum*** **Linn**.[29] and in ***Mangifera indica*** **Linn.**[30]. Kaur-16-ene, determined to be present only in 'Kasthuri' oil, is a diterpene. Its presence has been reported in a few volatile oils, including the flower volatiles of ***Clusia*** species[31]. A diterpene derivative 17-(acetyloxy)-kauran-18-al is also determined to be present in 'Kasthuri' oil only.

Conclusion

K. galanga varieties 'Kasthuri' and 'Rajani' differ morphologically as well as essential oils from their rhizomes have remarkably different physico-chemical characteristics and chemical compositions. Thus, the establishment of both the varieties is recommended for extraction of useful essential oils.

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