Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 1554-1558
Received: 13-07-2019
Accepted: 15-08-2019

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Evaluation of *Kaempferia galanga* L. morphotypes and its chemical markers

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Abstract
Kaempferia galanga is an important medicinal plant belonging to family Zingiberaceae. It forms a component of various Ayurveda medicines. Two morphotypes of *K. galanga* collected from Arunachal Pradesh and Kerala were evaluated for growth and yield. The volatile oil composition was investigated by Triple quadruple GCMSMS. Result showed the morphotype ArPCG-1 with higher number of tillers, leaves and plant spread. The morphotype KCG-1 exhibited higher growth and yield parameters i.e. leaf area, fresh and dry weight of leaves and higher girth of rhizome. In the GCMSMS analysis, a total of 27 compounds were identified from volatile oil of *K. galanga*. The dominant compounds identified were Eucalyptol (20.94 %), Ethyl p-methoxycinnamate (16.44%), Pentadecane (15.63 %), à-Pinene (12.76 %), Camphene (10.82 %).

Keywords: *Kaempferia galanga*, morphotypes, growth, yield, GCMSMS

Introduction
Kaempferia galanga popularly known as aromatic ginger or *Kacholam* originated in India and this highly priced medicinal plant is commercially cultivated in India, South East Asia and China. It is reported to have varying chromosome numbers viz. 2n=54 (Raghavan and Venkatasubban, 1943; Omanakumari and Mathew, 1985) [3, 1, 2], 2n=44 (Raghavan and Arora, 1958) [3], 2n=22 (Sharma and Bhattacharyya, 1959) [4]. The plant is a glabrous aromatic herb which forms a component of over 59 Ayurvedic medicines (Sivarajan and Balachandran, 1994) [5] and is extensively used in pharmaceutical industries for preparation of ayurvedic drugs, perfumery, and cosmetics and as spice ingredients (Rahman *et al.*, 2004) [6]. It is reported to possess anti-inflammatory property (Jagadish *et al*. (2016) [7], sedative activity (Ali *et al*. 2015) [8], antinociceptive activity (Ridtitid *et al*. 2008) [9], antidaibetic (Chowdhury *et al*., 2014) [10], antioxidant and antimicrobial (Rao and Kaladhar 2014) [11] activities.
Crop plants like *K. galanga* which is reproduced vegetatively has less chance of crop improvement than other crops as sexual reproduction is a constraint in this plant. In such species, collection and evaluation of morphotypes from diverse agro climatic conditions can be an alternate method to select a suitable material for the specific agro - ecological condition.

Materials and Methods
The present investigation was carried out at the Department of Plantation crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur during 2016-18. The evaluation was done for two consecutive years (2016-17 and 2018-19). The experiment material comprised of two morphotypes, one collection from Pasighat, Arunachal Pradesh (ArPCG-1) and another collection from Kerala (KCG-1). Just sprouted rhizomes of 25-35 g were used as planting material. They were planted in grow bags filled with mixture of soil, sand and FYM in equal proportions with the onset of south west monsoon. The grow bags were kept under shade net (50 % shade). Recommended dose of organic manure and other cultural package of practices were adopted for better crop growth as per ADHOC organic POP recommendations of KAU (2016). Drying up of leaves is the sign of maturity at 7-8 months. The rhizomes were dug out carefully, leaves removed and the rhizomes were cleaned off adhering soil and debris.
The volatile oil was analysed by Triple quadruple GCMSMS (Model TSQ 8000 MSMS). TG5M5 column (30 mm × 0.25 mm, 0.25 µm film thicknesses) was used as stationary phase. The oven temperature started from 60° C to 240° C with a constant rate of 3°C/min. The carrier gas was helium with the flow rate of 1 mL/min. One microliter of the oil (1:100 in HPLC grade methanol) was injected by Finnigan Autoinjector AI3000 with split ratio of 10:1. MS was performed by electron impact positive mode at 70 electron volts. The chemical constituents were identified by matching mass spectra and retention time indices with NIST MS Search 2.0 Library. Peak area was shown in percentage.

Results and Discussion

Morphology and yield

Table 1: Qualitative parameters in *K. galanga*

Parameters	Details
Rhizome colour: Scale Inner core	Dark reddish brown Pearl white
Rhizome shape	Globous
Presence of root tubers	Present
Colour of root tubers	Creamy white
Mature leaf colour	Dark green
Leaf tip shape	Acute
Growth habit	Sprawling

The qualitative characters did not show much variation among the two collections. Rhizome of were dark reddish brown in colour with pearl white inner core. Rhizome possessed tuberous roots also. Dark green leaves were round ovate with acute tip and entire margin. These qualitative features are in agreement with the reports of Aiyer and Kolammal (1964) [12] and Indrayan *et al*. (2007) [13].

Data given in table 2 indicate that in plant spread, ArPCG-1 showed significantly higher (29.16, 28.50) E-W spread. Similar findings of plant spread have been reported by Divya (2008) [14] in *K. galanga*.

Morphotype KCG-1 showed significantly higher leaf area during 2016-19. The variability in leaf area among the two collections might be due to the inherent character of the morphotypes. Latha (1994) [15] has reported variation in leaf area in *K. galanga* morphotype. Similar variations in leaf area have also been reported in turmeric by Krishna *et al*., (2019) [16].

Both tiller and leaf production were significantly higher in morphotype ArPCG-1 during both the years (Fig. 1). Variation in number of tillers was reported by Latha (1994) [15] and Divya (2008) [14] in *K. galanga*. In *Curcuma amada*, tillers produced per plant ranged from 1 to 4 among the nine accessions studied at Tsukuba, Japan (Jatoi *et al*., 2015) [17]. Variation in number of leaves may be explained as the result of the indirect influence of plant height and number of tillers as reported by Nybe (1978) [18] in ginger.

Fresh and dry weight of leaves was significantly higher in the morphotype KCG-1 when compared with ArPCG-1 during both the years. The morphotype recorded with higher leaf area also had higher fresh and dry weight of leaves.

Length of rhizome was slightly higher in the morphotype KCG-1 even though no significant difference was observed between the morphotypes (Table 3). In case of girth of rhizome morphotype KCG-1 recorded significantly higher values (21.33 mm, 20.7 mm) during both the years. Both morphotype showed the presence of root tubers. Number of tubers was significantly higher in the morphotype KCG-1 when compared with ArPCG-1 during both the years with mean value of 28.67 and 23.50 respectively. During 2018-19 morphotype KCG-1 had significantly higher (12.86 cm) tuber length. In case of girth of root tuber, morphotype KCG-1 recorded significantly higher value (9.05 mm, 8.50 mm) when compared with ArPCG-1 during both the years. Fresh and dry yield of rhizome showed no significant difference between the morphotypes in both the years. The highest fresh yield was 46.84 during 2017-18 and 55.23 during 2018-19. The same trend was observed in dry yield of rhizome also. The variation in yield and growth attributes among cultivars grown under same agro-ecological conditions could be attributed to the genetic factors (Aiyadurai 1966, Subharayadu *et al.* 1976 and Jalgaonker *et al*. 1988) [19, 20, 21]. Gayathiri and Anburani (2008) [22] reported the fresh weight of rhizomes (76.66 g/plant), and dry weight of rhizomes (75.16 g /plant) in *kacholam*. The root tubers are modified form of contractile roots where terminal part is swollen and form an egg shape (Ruamrunsgri, 2015) [23]. These root tubers stored carbohydrate that would be utilized by the plant for proper growth and development in the next season. Sereena *et al*. (2011) [24] have also observed the presence of club shaped root tubers in *K. galanga*.

It was evident from the growth parameters and rhizome characters that the morphotype collected from Kerala (KCG-1) possessed larger leaves and larger rhizomes when compared with the Arunachal Pradesh collection. Similar variation has been reported by Pandey and Dobhal (1993) [25] in ginger.

GCMSMS profile of *K. galanga* volatile oil

A total of 27 compounds were identified from volatile oil of *K. galanga* by GCMSMS (Table 4). Major compounds identified were Eucalyptol (20.94 %), Ethyl p-methoxycinnamate (16.44%), Pentadecane (15.63 %), à-Pinene (12.76 %), Camphene (10.82 %). Other compounds were present in traces. According to Raina and Abraham (2015) germplasm collections of *K. galanga* showed qualitative resemblances in composition with variation in the percentage of constituent compounds. The composition of rhizome oil of *K. galanga* in present study is in agreement with the findings Wong *et al*. (1992) [26], Rao *et al*. (2009) [27] and Mohanty *et al*. (2011) [28]. There are reports on major component of the oil of *K. galanga* as trans-ethyl-p-methoxycinnamate (Raina and Abraham 2015; Hasegawa *et al*., 2016; Li *et al*., 2017) [30, 31]. However, in the present study Eucalyptol (20.94 %) ranked first followed by Ethyl p-methoxycinnamate.

Conclusion

It was evident from the growth parameters and rhizome characters that the morphotype collected from Kerala (KCG-1) possessed larger leaves and larger rhizomes when compared with the Arunachal Pradesh collection. ArPG-1 recorded significantly higher plant spread, number of tillers and number of leaves while the morphotype KCG-1 had higher leaf area, fresh and dry weight of leaves. In GCMSMS profiling of volatile oil, compound Eucalyptol was found to the major constituent (20.94 %) followed by Ethyl p-methoxycinnamate (16.44%).

Table 2: Growth parameters in *K. galanga* morphotypes

Parameters	Year	Morphotypes	Mean
Plant spread (NS) (cm)	2016-17	KCG-1	28.50
		ArPCG-1	30.83
	2018-19	KCG-1	30.67
		ArPCG-1	32.17
Plant spread (EW) (cm)	2016-17	KCG-1	23.67
		ArPCG-1	29.16a
	2018-19	KCG-1	23.83
		ArPCG-1	28.50a
Leaf area (cm^2)	2016-17	KCG-1	144.03a
		ArPCG-1	131.76
	2018-19	KCG-1	147.27a
		ArPCG-1	131.89
Fresh weight of leaves (g/plant)	2016-17	KCG-1	96.17a
		ArPCG-1	60.00
	2018-19	KCG-1	91.0a
		ArPCG-1	62.33
Dry weight of leaves (g/plant)	2016-17	KCG-1	7.33a
		ArPCG-1	4.06
	2018-19	KCG-1	8.71a
		ArPCG-1	5.14

[a] indicates significant difference between two morphotypes for the given parameter. (t test, p=0.05, n=10)

Table 3: Rhizome characters in *K. galanga* morphotypes

Parameters	Year	Morphotypes	Mean
Length of rhizome (cm)	2016-17	KCG-1	8.87
		ArPCG-1	7.88
	2018-19	KCG-1	8.33
		ArPCG-1	7.90
Girth of rhizome (mm)	2016-17	KCG-1	21.33a
		ArPCG-1	14.92
	2018-19	KCG-1	20.7a
		ArPCG-1	16.16
No. of root tuber	2016-17	KCG-1	28.67a
		ArPCG-1	14.40
	2018-19	KCG-1	23.50a
		ArPCG-1	11.67
Length of root tuber (cm)	2016-17	KCG-1	13.78
		ArPCG-1	15.08
	2018-19	KCG-1	12.86a
		ArPCG-1	7.73
Girth of root tuber (mm)	2016-17	KCG-1	9.05a
		ArPCG-1	7.38
	2018-19	KCG-1	8.50a
		ArPCG-1	7.20
Fresh yield of rhizome (g/plant)	2016-17	KCG-1	46.12
		ArPCG-1	46.84
	2018-19	KCG-1	53.70
		ArPCG-1	55.23
Dry yield of rhizome (g/plant)	2016-17	KCG-1	21.45
		ArPCG-1	22.27
	2018-19	KCG-1	20.83
		ArPCG-1	25.33

[a] indicates significant difference between two morphotypes for the given parameter. (t test, p=0.05, n=10)

Table 4: GCMSMS profile of *K. galanga* volatile oil

Sl. No.	Compound	RT	Mol. Wt.	Area
1.	Ethanol, 2-(trimethylsilyl)-	2.09	118	0.86
2.	Camphene	7.62	136	10.82
3.	à-Pinene	10.20	136	12.76
4.	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	10.46	134	4.75
5.	Eucalyptol	10.97	154	20.94
6.	ç-Terpinene	11.65	136	0.01
7.	Farnesene epoxide, E-	13.53	152	0.07
8.	(+)-2-Bornanone	15.21	152	0.04
9.	endo-Borneol	16.85	154	5.54
10.	trans-2-Caren-4-ol	17.03	152	0.13
11.	Thymol	17.34	150	0.05
12.	L-à-Terpineol	17.63	154	0.07
13.	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	18.34	150	0.16
14.	Linalyl acetate	18.41	196	0.03
15.	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]	26.34	204	0.77
16.	Epizonarene	26.89	204	0.06
17.	2-Propenoic acid, 3-phenyl-, ethyl ester	28.89	176	8.15
18.	Pentadecane	31.37	212	15.63
19.	ç-Muurolene	31.82	204	1.24
20.	1-Carbethoxy-3-[à-hydroxy-á-N-phenylpiperazinoethyl]-4-[h]-quinolizine-4-one	31.95	421	0.10
21.	Cubenol	35.14	222	0.06
22.	Pyridine, 4-(4-methyl-5-cis-phenyl-1,3-oxazolidin-2-yl)-	41.23	240	0.11
23.	Ethyl 5-(5-methyl-2-furyl)-2,4-pentadienoate	41.79	206	0.15
24.	Ethyl p-methoxycinnamate	43.47	206	16.44
25.	Kaur-16-ene, (8á,13á)-	47.35	272	0.05
26.	Aristolene epoxide	51.61	220	0.03
27.	Cyclopenta[a,d]cycloocten-5-one, 1,2,3,3a,4,5,6,8,9,9a,10,10a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-4-met hylene	58.08	286	0.03

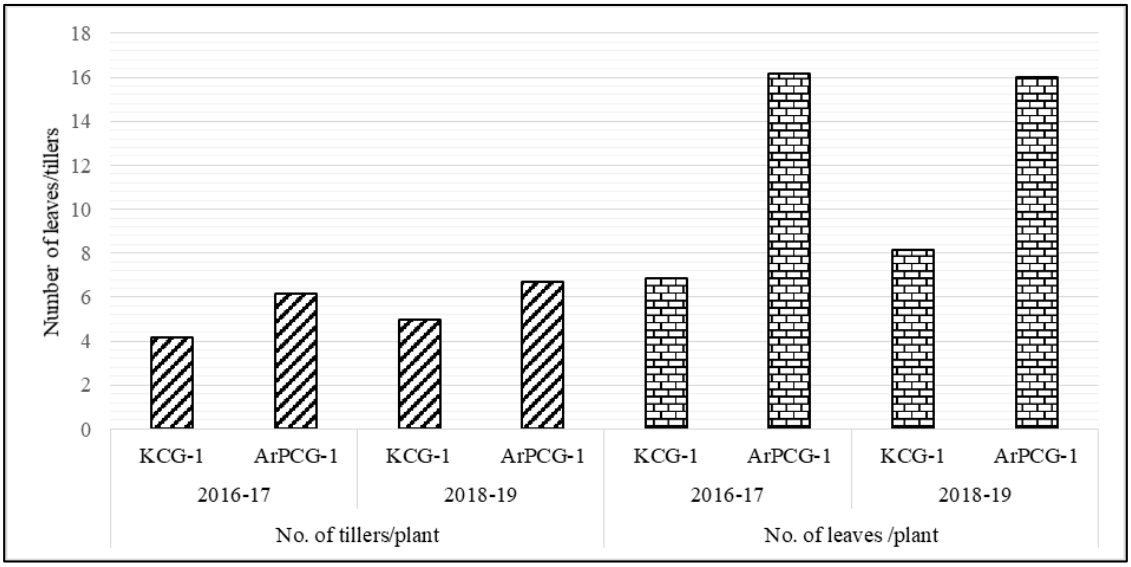

Fig 1: Number of tillers and leaves per plant of *K. galanga* morphotypes

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