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COMPARATIVE CHEMICAL PROFILING OF ESSENTIAL OIL COMPONENTS USING GC-MS IN MICRO, MINI AND MOTHER RHIZOMES OF *KAEMPFERIA GALANGA* L.

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ABSTRACT

Kaempferia galanga L. is a pharmaceutically and traditionally important medicinal plant. Its rhizomes are having potential demand in ayurvedic drug preparation. The study presented here reports the essential oil components analysed using GC-MS in three samples of *K. galanga* such as the microrhizomes raised through tissue culture technique, the mini rhizomes harvested from the field transferred tissue culture-derived plants and the mother rhizomes, which are the stock plants (control). Potent bioactive compounds like ethyl-p-methoxycinnamate, ethyl cinnamate, borneol and pentadecane were detected in the samples. The amount ethyl p- methoxycinnamate was very high in microrhizome (58.088%) and minirhizome oil (31.24%) as compared to *in vivo* mother rhizome. The amount of ethlyl cinnamate was 9.21% in microrhizome oil and it increased to 39.42% in minirhizome oil. Pentadecane recorded 1.482% in microrhizome and its amount increased to 4.49% in minirhizome oil, while the amount was noticed in *in vivo* mother rhizome. Borneol was analysed from micro (1.349%) and minirhizome oil (3.57%) samples but it was absent in mother rhizome. In our study, *in vitro* microrhizome and minirhizomes essential oil depicted superior quality in terms of the bioactive compounds and was on par with the mother rhizome oil. The *in vitro* production methodology developed here would help in the large scale production of pharmacologically important components from *K. galanga*.

Keywords: Essential oil, *Kaempferia galanga*, Microrhizomes, Minirhizomes, Mother rhizomes, GC-MS, Ethyl cinnamate, Ethyl p-methoxycinnamate.

1. INTRODUCTION

Kaempferia galanga is an endangered medicinal plant of family Zingiberaceae [1] locally called as *Chandramulika*, *Karchoor*, *sugandhvacha*, resurrection lily, and aromatic ginger. The species is mostly cultivated in south-east Asian countries viz. China, Malaysia, Thailand, Indonesia, and India. Leaves, rhizomes and root tubers are the medicinally important parts of the plant. Leaves are used for flavouring foodstuffs, preparing mouth washes and hair tonic, they are antinociceptive and antiulcerative [2, 3]. Rhizomes are the officially useful parts which are used for curing bronchitis, asthma, malaria, skin disease, wounds and spleenic disorders [4]. Moreover, they are constituents of a variety of Ayurvedic preparations like *Dasamularista, Valiyarasnadikasaya, kaccoradichurna, Asanaeladitaila* and *Valiyanarayanataila* [5]. In Indonesia and Malaysia, traditional herbal preparation, known as 'Makjun' and 'Jamu', are consumed frequently for beneficial health effect [6]. They are used for the preparation of decoction or powders, which are used for indigestion, cold, pectoral pain, abdominal pain, headache and toothache [7]. Thus the plant is an economically important species which forms the main ingredient of many ayurvedic drugs used for the healing of rheumatism [8] and the aromatic essential oil extracted from the rhizome is a valuable component of perfume production. The essential oil compounds were analyzed earlier in its rhizome by many workers and the plant contains 2.4 to 3.9% volatile oil [9, 10]. Essential oil from the rhizomes was found active against gram positive and gram negative microorganisms [11, 12] and hence it could be used for the skin infections and microbial diseases [13]. Also, it is a good natural source of a biologically active ester compound ethyl p-methoxycinnamate, which was found to exhibit anticancer activity [14]. Ethyl cinnamate and ethyl p-methoxycinnamate, are the most active compounds in essential oil of *K. galanga* [13, 15]. The price of the

essential oil of the plant varies from US $ 600 to 700 per kg on the international market, and this plant is over exploited by local people and pharmaceutical companies. The essential oil is used in Bangladesh for fragrance in vinegar, hair washes, cosmetic powders, flavoring the foodstuffs and beverages [16].

In addition, *K. galanga* plant has been reported to have broad-spectrum pharmacological and biological activities including antioxidant [17] larvicidal [18]), antibacterial [19], sedative [20], antineoplastic [21], vasorelaxant [22] and nematicidal [23] effects. Thus there are a number of reports on the bioactive potentialities, *in vitro* propagation and conservation of the species. As the rhizomes of the species are having potential demand in pharmaceutics, other routes of material production needs to be formulated to avoid further scarcity of the same due to over exploitation. Biotechnological methods using plant tissue culture technology have offered the production of *in vitro* clones in this taxa [24]. However, an analysis of the phytocomponents of the microrhizomes and minirhizomes that were derived from the *in vitro* procedures were not attempted yet. The present investigation reports the induction of microrhizomes, minirhizomes and comparison of chemical constituents in the essential oil of the same in *Kaempferia galanga*.

2. MATERIAL AND METHODS

2.1. Plant Material

Rhizomes of *Kaempferia galanga* L. collected from Kundara, Kollam District, Kerala State, India, was used as the plant material for the *in vitro* microrhizome induction and essential oil extraction.

2.2. Production of micro and mini rhizomes

Rhizomes containing axillary buds were inoculated aseptically in MS medium containing 0.5mgl^{-1} BA according to the standardized procedure [25]. Initiated shoots were multiplied in MS medium augmented with 3.0 mgl^{-1} BA and 0.5mgl^{-1} NAA. For *in vitro* microrhizome induction, the *in vitro* shoots established in 3.0 mgl^{-1}BA and 0.5 mgl^{-1} NAA were then subcultured to the fresh medium of the same composition augmented with different concentration of silver nitrate (1.0 mgl^{-1} and 2.0 mgl^{-1}) and sucrose (3, 6 and 9%) (w/v). After six months, the *in vitro* microrhizomes were collected and washed in running tap water to remove all the remnants of culture medium and were used for essential oil extraction. The *in vitro* plantlets were planted in polythene bags filled with garden soil and river sand (3:1) and maintained in the green house and regular watering for the production of minirhizomes. After six months period, the minirhizomes were collected for further analysis.

2.3. Extraction of essential oil

Twenty five grams each of microrhizomes collected from the culture vessel, minirhizomes collected from the green house and *in vivo* mother rhizomes collected from the field (control) were subjected to hydrodistillation using a modified Clevenger-type glass apparatus for 4hours for isolation of essential oils separately.

2.4. GC-MS analysis

The GC-FID analysis was carried out on a Varian CP-3800 gas chromatograph equipped with flame ionaisation detector (FID) and a CP Sil 8CB fused silica capillary column (30 m×0.32 mm, film thickness-0.25 mm). Nitrogen was used as carrier gas at flow rate 1ml/min. The oil constituents were identified by MS library search (WILEY 275), comparison of the relative retention indices were calculated with respect to homologous of n-alkanes (C_6-$C_{30,}$ Aldrich Chem. Co. Inc) [26] and comparison of mass spectrum reported in the literature [27].

3. RESULTS AND DISCUSSION

Essential oils collected from *in vivo* rhizomes (control), *in vitro* microrhizomes and minirhizomes of *K. galanga* analysed by GC-MS (Figs. 1-3) and the components present were presented in Tables 1-6. As per GC-MS analysis, there were 79 components in *in vivo* rhizome oil. The major compounds detected in the mother rhizomes and their reported bioactivities are given in Tables 1 and 2. Palmitic acid (12.23%) was detected as the predominant compound in addition to capric acid (8.96%), caprylic acid (4.47 %), lauric acid (3.08 %), myristic acid (3.92 %), stearic acid (3.95%), ethyl-p-methoxycinnamate (2.13%) and ethyl cinnamate (0.79%). In *in vitro* microrhizome oil, there were 74 compounds and ethyl p-methoxycinnamate (58.088%) was detected as most abundant component. Remaining compounds were ethyl cinnamate (9.16%), Octahydro-4a (2H)-naphthalinyl methanol (2.05%), borneol (1.35%), pentadecane (1.48%), α-cadinol (0.73%) and Retinol (0.50%) (Table 2) and minirhizome oil of *K. galanga* containing 15 major compound. In minirhizome oil, ethyl cinnamate (39.42%) was the abundant constituent and ethyl p-methoxycinnamate (31.24 %) was also present in an appreciable amount. The other

compounds recorded in minirhizome oil of *K. galanga* are α-pinene (1.16%), Camphene (1.02), M-Cymene, D-Limonene (0.9%), Eucalyptol (5.30), Borneol (3.57%), Germacrene (1.09%) and Pentadecane (4.49%).

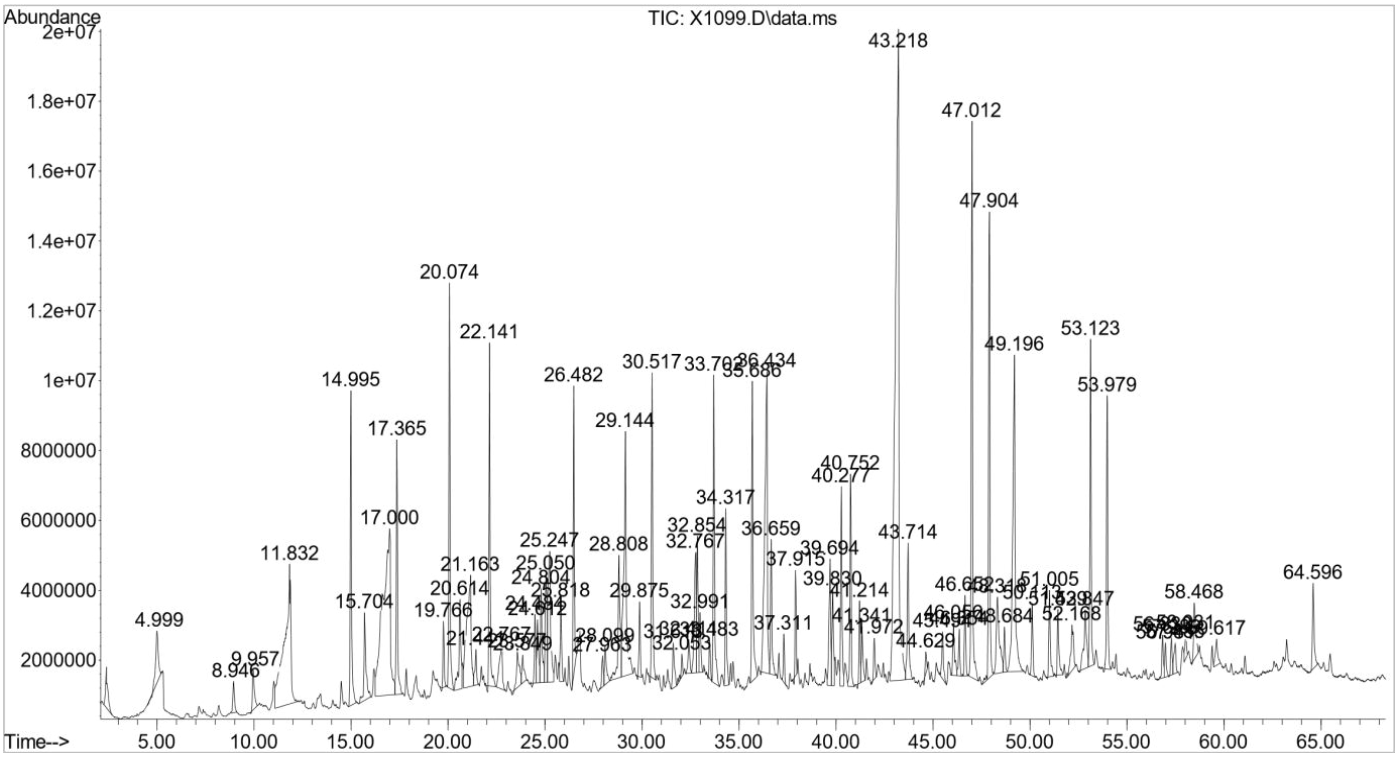

Fig. 1: GC-MS chromatogram of *in vivo* mother rhizome oil of *K. galanga*

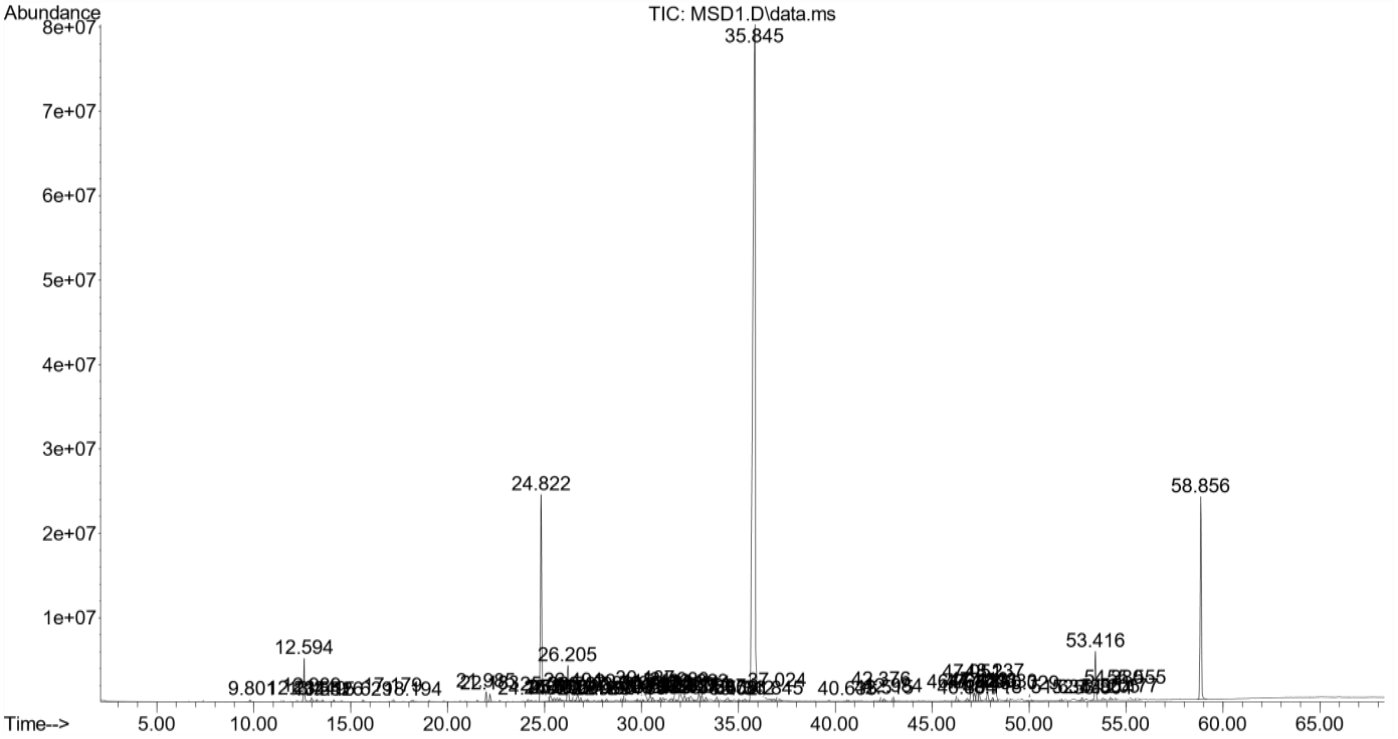

Fig. 2: GC-MS -Chromatogram of *in vivo* microrhizome oil of *K. galanga*

Table 1: Essential oil components in *in vivo* mother rhizome of *K. galanga*

Peak number	Retention time	Compound	Common name	Chemical formula	Abundance (%)
1	2.39	1-octanol	Octylalcohol/fatty alcohol	$CH_3(CH_2)_7OH$	0.44
2	4.99	Nonanal	Nonanaldehyde	$C_9H_{18}O$	0.69
5	11.83	0ctanoic Acid	Caprylic Acid	$C_8H_{16}O_2$	4.47
6	14.99	2-Decenal	Aldehyde	$C_{10}H_{18}O$	2.47
7	15.70	Cyclododecane	Cyclododecane	$C_{12}H_{24}$	0.90
8	17.00	n-Decanoic acid	Capric acid	$CH_3(CH_2)_8COOH$	6.45
9	17.36	Tridecanal	Saturated fatty acid	$C_{13}H_{26}0$	1.72
11	20.07	2-Dodecenal	Unsaturated fatty aldehyde	$C_{12}H_{22}O$	2.47
12	20.61	1-Undecanol	Fatty alcohol	$C_{11}H_{24}O$	0.92
13	21.16	n-Decanoic acid	Capric acid	$CH_3(CH_2)_8COOH$	2.51
15	22.14	Cyclododecane	Cycloalkane	$C_{10}H_{20}$	2.02
16	22.77	2-decenoic acid	Monosaturated fatty acid	$C_{10}H_{18}O_2$	0.68
19	24.48	2(3H) Furanone-	ɣ- Decalactone	$C_{10}H_{18}O_2$	O.43
20	24.61	2-Dodecenal	Unsaturated fatty aldehyde	$C_{12}H_{22}O$	0.56
21	24.80	2-propenoic acid ,3-phenyl,ethyl ester	Ethyl cinnamate	$C_{11}H_{12}O_2$	0.79
22	25.05	n-Tridecan-1-ol	Tridecanol	$C_{13}H_{28}O$	0.99
23	25.25	Undecanoic acid	Undecylic acid (carboxylic acid)	$C_{11}H_{22}O_2$	1.77
25	26.48	Tetradecanal	Myristyl aldehyde(myristic acid)	$C_{14}H_{28}O$	1.63
28	28.81	2(3H)Furanone,5-heptyl dihydro-	ɣ-Undecalatone	$C_{11}H_{20}O2$	1.32
29	29.14	Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	3.08
31	30.52	Tetradecanal	Myristyl aldehyde(myristic acid)	$C_{14}H_{28}O$	1.97
35	32.77	Tridecanoic acid	Tridecylic acid(saturated fatty acid)	$C_{13}H_{26}0_2$	1.17
36	32.85	2(3H)Furanone,5-heptyl dihydro	ɣ-Undecalatone	$C_{11}H_{20}O2$	0.92
39	33.70	2-pentadecanone	Methyl tridecyl ketone	$C_{15}H_{30}O$	2.09
40	34.32	Hexadecanl	Palmityol	$C_{16}H_{32}O$	1.14
41	35.69	2-Propenoic acid,3(4-methoxyphenyl)-ethyl ester	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	2.13
42	36.43	Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	3.92
43	36.66	1-Decanol, 10[(tetrahydro-2H-pyran-2-yl)oxy]-	1-Decanol, 10[(tetrahydro-2H-pyran-2-yl)oxy]-	$C_{15}H_{30}O_3$	0.98
46	39.69	Pentadecanoic acid	Pentadecanoic acid(saturated fatty acid)	$C_{15}H_{30}O_2$	1.08
48	40.28	Sulfurous acid, hexyl undecyl ester	Hexyl undecyl sulphite	$C_{17}H_{36}O_3S$	1.35
49	40.75	2-Heptadecanone	Methyl pentadecyl ketone	$C_{17}H_{34}O$	1.37
53	43.22	n-Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	12.22
54	43.71	2-(6-bromohexyloxy) tetrahydro-2H pyran	2-(6-bromohexyloxy) tetrahydro-2H pyran	$C_{11}H_{21}BrO_2$	1.09
60	47.01	2(3H)-Furanone,5-dodecyldihydro-	Gamma palmitolactone	$C_{16}H_{30}O_2$	3.92
61	47.90	RH-Pyran-2-one,tetrahydro-6-nonyl	δ-tetradecalactone	$C_{14}H_{26}O_2$	3.26
62	48.38	Oleic acid	Oleic acid	$C_{18}H_{34}O_2$	1.19
64	49.19	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	3.95
69	52.85	6-Octadecenoic acid(z)-	Petroselinic acid	$C_{18}H_{34}O_2$	0.82
70	53.12	2(3H)-Furanone,dihydro-5-tetradecyl	ɣ-Stearolactone	$C_{18}H_{34}O_2$	2.11
71	53.98	2sH-Pyran-2-one,tetrahydro-6-tridecyl	δ-Octadelactone	$C_{18}H_{34}O_2$	1.80
75	57.49	Pentadecane	Pentadecane	$C_{15}H_{32}$	0.32

Table 2: Major compounds in *in vivo* mother rhizomes and their activities

Compound	Bioactivity
Palmitic acid	Antifungal, antibacterial, antiinflammatory, production of cosmetics, soaps, etc. [29].
Capric acid	Antimicrobial, larvicidal, cholesterol dissolving agent, manufacture of perfumes [30].
Caprylic Acid	Antifungal, antibacterial [20], Cleansing agent, perfuming agent
Lauric acid	Antimicrobial, used in alcoholic beverages, flavour and fragrance agent,
Stearic acid	Anticancerous, production of cooking oil, soap cosmetics, soap production [36].
Myristic acid	Anti-microbial, Improving HDL [37], making cosmetics, fragrance ingredient
Gamma palmitolactone	Antimicrobial activity, flavouring agent
δ-tetradecalactone	Flavour ingradient
Ethyl p-methoxycinnamate	Antiinflammatory [15], antifungal [38], larvicidal [39], analgesic, anti-iv, TB molecule [40], Cytototoxic pro and pro-apoptotic properties, Anticancer [14]
Ethyl cinnamate	Anticancer [41], antimicrobial, anti-oxidative [42], Mosquito repellent activity, Vasorelaxant activity [22], Larvicidal activity [43].
Pentadecane	Antibacterial [44].

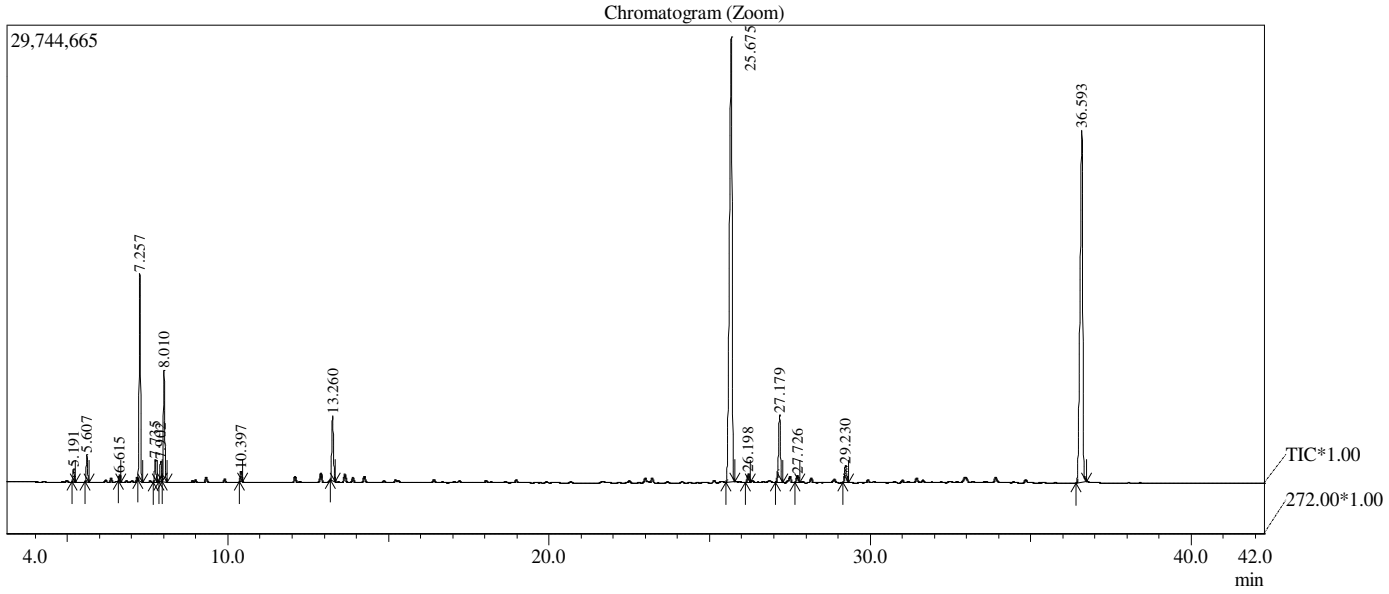

Fig. 3: GC-MS -Chromatogram of *in vivo* minirhizome oil of *K.galanga*

3.1. Major compounds in *in vivo* rhizome oil and their bioactivities

Most of the compounds found in the samples analysed here have been reported to exhibit significant biological activities. Some of the major compounds in *in vivo* rhizome oil and their bioactivities were mentioned in Table 2. According to scientists [28] monoterpenes and sesquiterpenes were found in the essential oil of *K. galanga* rhizomes which have contributed the flavour and fragrance properties to the oil. Among the 41 compound analysed in *in vivo* rhizome oil, the highest percentage of abundance was with palmitic acid (12.22), a saturated fatty acid which is having many medicinal properties like antifungal, antibacterial and also used for the production soaps and cosmetics [29]. Capric acid (6.45%), was another fatty acid component present in *in vivo* rhizome essential oil of *K. galanga*. This is also a saturated fatty acid with anti inflammatory and antibacterial properties [30]. These fatty acids were also present in many other zingiberaceous members. For example, 22.18% palmitic acid and 3.81% of capric acid was reported in *Z. officianale* [31].

Caprylic acid, one of the compounds present in *in vivo* rhizome oil is used commercially in the production of esters used in perfumery and also in the manufacture of dyes. Caprylic acid has been studied as part of a ketogenic diet to treat children with intractable epilepsy [32] and is currently being researched as a treatment for essential tremor [33]. Its presence is also reported in Zingiberaceae members like *Curcuma longa* [34] and *Z. officianale* [31]. Lauric acid, Stearic acid and myristic acids are also present in *in vivo* rhizome oil.

Quantification of these components were previously done [31] in *Z. officianale* (Lauric acid 8.34%, Stearic acid 3.45%, myristic acid 5.85%) and in *Heliotropium bacciferum* [35] (Stearic acid 1.74% and myristic acid 0.20%). Ethyl p-methoxycinnamate and ethyl cinnamate were the most active compounds in *K. galanga* based on previous studies [5]. However, these compounds were also analysed in mother rhizome oil samples in least amount, but interestingly, the quantity of these medicinally important compounds were in enhanced levels in the microrhizome and minirhizome oil samples.

3.2. Major compounds in *in vitro* microrhizomes and their bioactivities

Bioactive compounds present in *in vitro* microrhizome oil of *K. galanga* were explained in the Table 3. Major compounds present in *in vitro* rhizome oil and their bioactivities were mentioned in Table 4. The present study revealed that, there is great variation in the case of chemical constituents in *in vivo* rhizome and *in vitro* microrhizome. The percentage of ethyl p-methoxycinnamate and ethyl cinnamate was comparatively high in *in vitro* microrhizome than *in vivo* rhizome. In *in vitro* rhizome oil, ethyl p-methoxycinnamate (58.088%) was the predominant compound. The major compounds present viz. ethyl cinnamate and ethyl-p-methoxycinnamate are esters which contribute the nematicidal, anticancer, antituberculosis, anti-inflammatory, antifungal and larvicidal properties [39] to the oil. It has been used for the treatment of pain and inflammation and this compound also exhibits inhibitory activity against proliferation of tumor cell in the specimen of mouse epidermis and extent of papilloma [45]. Based on the previous reports [10] rhizome oil of *K. galanga* contain 46.81% while, the *in vitro* whole plant oil contain 51.23% of ethyl p- methoxycinnamate. Here in our sample i.e., *in vitro* microrhizome essential oil contain predominantly high amount of (58.088) ethyl p-methixycinnamate. Another active compound present was ethyl cinnamate (9.78%) having many reported medicinal properties like anticancer [41], antimicrobial, anti-oxidative [42], mosquito repellent and vaso-relaxant activity [22]. Borneol (1.349%) was seen in *in vitro* rhizome oil, while 2.85% borneol was detected in dried conventional rhizome samples of *K. galanga* [13] and 1.0-2.4 % borneol was reported in *K. galanga* by [46]. It is a terpene derivative having many medicinal properties like, anticoagulant and antithrombotic activities [47] along with antihypertensive and anti-oxidant activities [48]. Pentadecane is one of the active compound reported in *K. galanga* and 1.48% of pentadecane was present in *in vitro* microrhizome essential oil. According to scientists [10], the *in vitro* whole plant rhizome oil contains 3.69% pentadecane and in conventional rhizome oil, it was 5.81%. Humulene epoxide 2, retinol, caryophyllene, α-cadinol are the other active compounds present in *in vitro* rhizome oil. α-cadinol is a cadinane sesquiterpenoid having antifungal activity [49] and it was previously reported in *K. galanga* by [10], it was 0.11% in rhizome oil and 0.06% *in vitro* whole plant oil and in our sample 0.71% of cadinol is recorded. Caryophyllene and humulene epoxide 2 were also sesquiterpenoids, reported earlier in *K. galanga* [50] and 0.41% of caryophyllene and 0.61% humulene epoxide were present in the microrhizome oil of *K. galanga*. Humulene is also known as α -caryophyllene and it is having many therapeutical and pharmacological applications [51]. Retinol is also present in *in vitro* microrhizome oil of *K. galanga,* it is also called vitamin A_1; vitamin in the vitamin A family and used as a dietary supplement. As a supplement it is used to treat and prevent vitamin A deficiency [52].

3.3. Major compounds in minirhizomes and their activities

GCMS analysis of *K. galanga* minirhizome essential oil, revealed the presence of 15 major compounds. Among these, ethyl p-methoxycinnamate (31.24%) and ethyl cinnamate (39.42%) were the predominant ones. Eucalyptol (5.30%) a monoterpenoid, was one of the compound present and its presence already reported in other zingiberaceae species like *Hedychium flavescens* (15.3%) [54] and 2.12% in *K. galanga* [55]. Borneol is a terpene derivative with many medicinal properties like insect repellent, traditional Chinese medicine, skin tonic, sedative and antiplasmodic, stimulating the production of gastric juices, improve circulation, treat bronchitis, reduce swellings, relives stress and has analgesic potential [53]. The minirhizomes recorded 3.5% of borneol and the same amount was reported earlier [46], while the amount was lesser (1.03%) [55]. Camphene (1.36%), α-pinene (0.52%), Carene (9.52%), pentadecane (4.49%),germacrene (1.09%) were the other constituents present minirhizome oil of *K. galanga*.

Table 3: Essential oil components in *in vitro* microrhizome of *K. galanga*

Peak number	Retention time	Compound	Common name	Chemical formula	Abundance (%)
3	12.23	Borneol	Borneol	$C_{10}H_{18}O$	1.35
4	12.99	Benzene methanol α, α,4-trimethyl	P-Cymenol	$C_{10}H_{14}0$	0.22
9	17.18	Bornyl acetate	Acetate ester of borneol	$C_{12}H_{20}O_2$	0.20
14	24.82	2-propenoic acid ,3-phenyl,ethyl ester	Ethyl cinnamate	$C11H12O_2$	9.77
19	26.20	Pentadecane	Pentadecane	$C_{15}H_{32}$	1.48
20	26.49	Naphthalene ,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	α -amorphene	$C_{15}H_{24}$	0.38
21	26.71	Naphthalene ,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1s-cis)-	α -Cadinine	$C_{15}H_{24}$	0.36
27	29.12	Caryophyllene oxide	Caryophyllene oxide	$C_{15}H_{24}O$	0.34
30	30.19	12-Oxybicyclo[9.1.0] dodeca-3,7diene,1,5,5,8-tetramethyl	Humulene epoxide 2	$C_{15}H_{24}O$	0.63
31	30.45	Dihydro-cis-alpha-copaene-8-ol		$C_{15}H_{26}O$	0.41
37	31.98	α-Cadinol	Cadinanesesquiterpenoid	$C_{15}H_{26}O$	0.73
38	32.22	Cyclohexane,6-ethenyl-6-methyl-1-(1-methyl)-3-(1-methylethylidene-(s)-	Alpha elemene	$C_{15}H_{24}$	0.33
40	32.52	Caryophyllene	Bicyclic sesquiterpene	$C_{15}H_{24}$	0.41
41	32.96	1,E-8,Z-10-Tetradecatriene		$C_{16}H_{28}$	0.51
42	33.11	Aromadendrene, dehydro	Dehydromadendrene	$C_{15}H_{22}$	0.33
45	34.46	(E)-3(10)-Caren-4-ol		$C_{10}H_{16}O$	0.28
48	35.85	2-Propenoic acid,3(4-methoxyphenyl)-ethyl ester	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	58.09
52	42.38	1-Buten-1-ol,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen0.-1-yl)-		$C_{13}H_{22}O$	0.43
55	46.27	Benzene,1,1-[1-(methylthio)ethylidene]bis-	1-Methylsulfanyl-1-(phenylethyl)benzene	$C_{15}H_{16}S$	0.35
57	47.05	(z)-4-Chloro-2,3-dimethyl-1,3-hexadiene		$C_8H_{13}Cl$	1.18
58	47.18	Anthracene,9-propyl-	9-Propylanthracene	$C_{17}H_{16}$	0.37
59	47.32	Retinol	Retinol	$C_{20}H_{30}O$	0.50
60	47.41	2,4-Bis(chloromethyl) mesitylene		$C_{11}H_{14}Cl_2$	0.49
61	47.85	3-Pyridinemethanol,5-hydroxy-4,6-dimethyl-		$C_8H_{11}NO_2$	0.44
68	53.42	4a(2H)-Naphthaleneme-thanol, octahydro		$C_{11}H_{20}O$	2.05
71	54.38	1-Ethyl-3-propyl-5-(propene-1-yl)adamantine		$C_{18}H_{30}$	0.51
72	55.18	Vitamin A acetate	Vitamin A acetate	$C_{22}H_{32}O_2$	0.25

Table 4: Major compounds in *in vitro* rhizomes and their activities

Compound	Bioactivity
Ethyl p-methoxycinnamate	Anti-inflammatory [15], antifungal [38]. larvicidal [39] analgesic ,anti-HIV, TB molecule [40], Cytototoxic pro and pro-apoptotic properties, Anticancer [14].
Ethyl cinnamate	Anticancer [41], antimicrobial, anti-oxidative [42], Mosquito repellent activity, Vaso-relaxant activity [22], Larvicidal activity [43].
Borneol	Insect repellent, traditional Chinese medicine, skin tonic, sedative and antiplasmodic, stimulating the production of gastric juices, improve circulation, treat bronchitis, reduce swellings, relives stress, Analgesic potential [53], Anticoagulant and antithrombotic activities [47], Antihypertensive and anti-oxidant [48]
Pentadecane	Antibacterial [44].
α-Cadinol	Antifungal [49], Hepatoprotective
Humulene epoxide 2	Used in alcoholic bevarages, present in many food items.
Retinol	Used to treat and prevent Vitamin A deficiency, stimulate blood flow and collagen production.
Caryophyllene	Inhibit cancer cell growth, reducing stress, slowing the growth of bacteria, reducing chronic inflammation.

Table 5: Essential oil components in minirhizome of *K. galanga*

Peak number	Retention time	Compound	Common name	Chemical formula	Abundance (%)
1	5.1	Bicyclo[3.1.1]Hept-2- Ene,2,6,6-trimethyl	α-pinene	$C_{10}H_{16}$	0.52
2	5.61	Bicyclo[2.2.1]Heptane,2,2-Dimethyl-3-Methyl	Camphene	$C_{10}H_{16}$	1.16
3	6.62	1,6- Octadiene, 7-Methyl-3-Methylene	Myrcene	$C_{10}H_{16}$	0.28
4	7.26	Bicyclo[4.1.0]Hept-3,7,7-Trimethyl	(+)-2Carene	$C_{10}H_{16}$	9.52
5	7.74	Benzene,Methyl(1-Methylethyl)-	M-Cymene	$C_{10}H_{14}$	1.02
6	7.90	D-Limonene	D-Limonene	$C_{10}H_{16}$	0.9
7	8.01	2-Oxabicyclo[2.2.2] Octane,1,3,3-Trimethyl	Eucalyptol	$C_{10}H_{18}O$	5.30
8	10.39	(5E,8E)-5,8,10-Undecatrien-3-ol		$C_{11}H_{18}O$	0.50
9	13.26	Bycyclo[2,2.1]Heptan-2-ol,1,7,7-Trimethyl	Borneol	$C_{11}H_{12}O$	3.57
10	25.68	2-propenoic acid ,3-phenyl, ethyl ester	Ethyl cinnamate	$C_{11}H_{12}O_2$	39.42
11	26.19	1,6 Cyclodecadiene,1-Methyl-5-Methylene	Germacrene		0.50
12	27.18	Pentadecane	Pentadecane	$C_{15}H_{32}$	4.49
13	27.73	Naphthalene,1,2,3,5.6.8A-Hexahydro-47 Dimethyl		$C_{15}H_{24}$	0.42
14	29.23	Germacrene B	Germacrene	$C_{15}H_{24}$	1.09
15	36.59	Ethyl-p- methoxycinnamate	Ethyl-p-methoxycinnamate	$C_{11}H_{14}O_3$	31.24

3.4. Comparison of major compounds in micro, mini and *in vivo* mother rhizomes

Common compounds present in the three essential oil rhizome samples i.e., *in vitro* microrhizome oil, minirhizome oil and *in vivo* mother rhizome oil and their percentage of abundance were plotted in Fig. 4. Ethyl p-methoxycinnamate, ethyl cinnamate, pentadecane were present in the three samples while borneol was recorded in micro and minirhizome oil. These three are the most active compounds in *K. galanga*. In our study we discussed about the comparison of three rhizome samples of *K. galanga*. Quantification of essential oil components from different part of *K. galanga* like leaves, rhizome, whole pants were carried out previously [7, 10, 46]. But, no studies yet were found regarding the esssential oil composition in microrhizome and minirhizome oil. Ethyl p-methoxycinnamate and ethyl cinnamate are found to be most vital constituents responsible for the pharmacological activities of *K. galanga*. Among the three samples analysed here, *in vitro* micro rhizome oil possess more amount of ethtyl p-methoxycinnamate i.e., 58.088% and is more or less similar to that of the report [10]. It's amount was 31.24% in minirhizome oil, while it was 2.132% in *in vivo* mother rhizome oil.

Table 6: Major compounds in minirhizomes and their activities

Compounds	Bioactivity
α-pinene	Anti-inflammatory and anti-carcinogenic effects [56]; larvicidal activity [57].
Camphene	Antifangal, antibacterial [58].
+)-2Carene	Air freshners, cosmetics
Eucalyptol	Insecicidal. Insect repellent, Cough supressent[59]
Borneol	Insect repellent, traditional Chinese medicine, skin tonic, sedative and antiplasmodic, stimulating the production of gastric juices, improve circulation, treat bronchitis, reduce swellings, relives stress, Analgesic potential [53] Anticoagulant and antithrombotic activities [47], Antihypertensive and anti-oxidant ([48]
Ethyl cinnamate	Anticance[r41], antimicrobial, anti-oxidative [42], Mosquito repellent activity, Vasorelaxant activity [22], Larvicidal activity [43]
Pentadecane	Antibacterial [44]
Germacrene	Anti-inflammatory, anti-bacterial, antifungal.
Ethyl-p- methoxycinnamate	Anti-inflammatory [15]antifungal [38] larvicidal [39] analgesic,anti-HIV, TB molecule [40], Cytototoxic pro and pro-apoptotic properties, Anticancer [14]

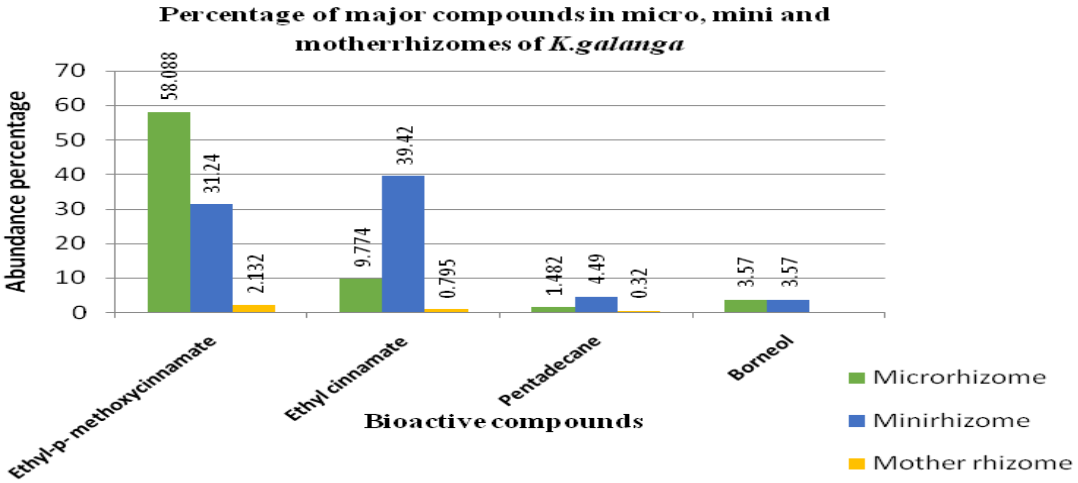

Fig. 4: Comparison of major compounds in *K. galanga* rhizome samples

The amount ethyl p- methoxycinnamate was very high in microrhizome and minirhizome oil as compared to the report [46] as well as with the findings [55] who previously proposed the amount of ethyl p-methoxycinnamate as 25.96% in essential oil *K. galanga*. Another potential component ethlyl cinnamate was 9.21 % in microrhizome oil and it increased to 39.42% in minirhizome oil. Earlier reports suggest 24.8% of ethy cinnamate in rhizome oil and 19.23% in i*n vitro* whole plants of *K. galanga* [10]. The amount of the same constituent in minirhizome oil in our analysis was much higher than that of earlier record of 11.5-26.6% [46]. According to scientists [17] the amount of ethyl cinnamate in conventional rhizome of *K. galanga* was 9.69% and its amount in *in vitro* rhizome grown in soil was 18.14%. Pentadecane; another common compound present in three samples recorded 1.482% in microrhizome and its amount is increased to 4.49% in minirhizome oil, while least amount was obtained from *in vivo* mother rhizome. The amount of pentadecane is comparable to the report [17] wherein the conventional rhizome oil contain 0.57% and *in vitro* rhizome grown in the soil has 1.46% of pentadecane. Here borneol was analysed from micro (1.349%) and minirhizome oil (3.57%) samples and it was absent in mother rhizome. However, the amount borneol in our samples are high when compared to the studies [55] that recorded 1.03% of borneol in essential oil of *K. galanga*.

4. CONCLUSION

The active constituents of *Kaempferia galanga* essential oil are ethyl p-methoxycinnamate, ethyl cinnamate, pentadecane and borneol. Here these compounds were detected from the essential oil samples of *in vitro* microrhizome and *in vivo* mother rhizomes and the percentage of abundance of ethyl p- methoxycinnamate was highest in microrhizome oil. Ethyl cinnamate was maximum in minirhizome oil than the other two samples. In our study, *in vitro* microrhizome and minirhizome essential oil depicted superior quality in terms of the bioactive compounds which is on par with the mother rhizome oil. Thus the findings established here offer the development of a novel method for the extraction of volatile oils from microrhizomes and minirhizomes. This system can be extended for future explorative approaches for drug preparations utilizing the microrhizomes and minirhizomes thereby shortening the gap of demand-supply in the phyto-industrial market.

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Conflicts of interest

There is no conflict of interest

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