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## INVESTIGATIONS ON *KAEMPHERIA GALANGA*

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**Abstract** : The constituents of rhizome oil and root oil of *Kaempferia galanga* are similar in GLC studies. Ethyl cinnamate and ethyl *p*-methoxy cinnamate are the major constituents. The volatile oil content of the rhizome is higher than that of the root. The optimum time to harvest the rhizomes is at the age of one year based on essential oil content. Higher yield is obtained in plants grown under coconut. The root and rhizome oils of *Kaempferia galanga* showed marked activity against *Staphylococcus aureus* and *Escherichia coli*

**Key words**: Antimicrobial activity, GLC analysis, *Kaempferia galanga*, volatile oils

## INTRODUCTION

*Kaempferia galanga* belongs to family Zingiberaceae and is distributed throughout the tropics and sub tropics of Asia and Africa. The rhizomes are traditionally used in medicine for the treatment of cough, pectoral infections<sup>1,2</sup>, abdominal pain<sup>1</sup>, malaria<sup>1</sup>, heart dyspepsia, depression, fever, rheumatism, sore throat, sore eyes and wounds. Antimalarial, antispasmodic and antitumour activities too have been reported.<sup>1,3-4</sup> The roots have shown antibacterial activities. The rhizomes are also used in perfumes and cosmetics. About 40 compounds including ethyl *p*-methoxycinnamate, and *p*-methoxy cinnamic acid have been reported in the volatile oil of *K. galanga* rhizomes found in other countries.<sup>2, 5-6</sup>

The present studies deal with the composition of the volatile constituents, the antimicrobial activities of the rhizome and root oils of *K. galanga* as well as determining suitable conditions for cultivation and the optimum time for harvesting the plants.

## METHODS AND MATERIALS

*Plant material*: *Kaempferia galanga*. L. ( Zingiberaceae) rhizomes and the roots were collected from plants grown in Colombo and Walpita and identified at the

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Botanical Gardens, Peradeniya. A voucher specimen is available in the herbarium in Peradeniya.

*Determination of the chemical constituents of the plant:* Samples of rhizomes and roots were collected from 1, 1 1/2 and 2 yr. old plants. The crushed rhizomes and roots were separately water distilled for 5 h. using a Clevenger arm and the volatile oil was collected. The yields were estimated on a dry weight basis. The oil was analysed by Gas Liquid Chromatography using Shimadzu GC 8A equipped with a FI detector and a 10% Carbowax 20M column. Retention data, peak enhancement techniques and GC-MS data were used for identification of compounds

**Table 1: Effect of maturity on the yield of volatile oils of *K. galanga***

Age of the Plant (Year)	Yield of oil (%)	
	Rhizome	Root
1	7.28±0.43	0.8±0.06
1 1/2	3.83±0.72	1.32±0.5
2	4.4±0.28	1.72±0.16

**Table 2: Variation in major constituents of the essential oil of *K. galanga* rhizome with maturity**

Compound	Yield%		
	1 Year	1 1/2 Years	2 Years
Camphene	0.5	0.6	3.5
1,8 cineol	0.4	1.5	3.0
Camphor	10.7	3.1	3.8
Borneol	2.7	2.7	3.8
Cinnamaldehyde	0.23	0.8	0.7
Ethyl Cinnamate	25.3	35.0	40.0
Quinazoline-4 phenyl -3-oxide	0.2	0.2	0.03
Ethyl <i>p</i> -methoxy cinnamate	48.9	39.0	32.7

**Table 3: Variations in major constituents of the *K. galanga* root oil with maturity**

Compound	Yield%		
	1 Year	1 1/2 Years	2 Years
Camphene	0.5	2.2	1.6
1,8 cineol	0.5	2.2	2.2
Camphor	2.4	2.1	2.8
Borneol	8.0	5.0	6.1
Cinnamaldehyde	0.4	1.6	1.9
Ethyl cinnamate	16.3	17.1	18.7
Quinazoline-4 phenyl -3-oxide	0.4	0.9	0.3
Ethyl <i>p</i> -methoxy cinnamate	25.8	37.8	40.9

*Evaluation of the performance of K. galanga grown under coconut and in open conditions:* This study was carried out at Walpita farm in Kotakadeniya. Age of the coconut plantation was 46 years and available sunlight was approximately 60% underneath the coconut which was planted at 8.5m x 8.5m spacing. The soil was moderately deep gravel. The *K. galanga* plants were established in 4m x 6m size plots prepared at the center of the coconut square. Plots were laid in Complete Randomized Block Design with three replications. Initially 500 kg of super phosphate was added to each plot. This was followed by 10 kg of dried cattle manure to each plot once in every three months. Another set of *K. galanga* plants were established in an open area (without coconut). The other management practices were similar. Growth and reproductive parameters were recorded monthly and the assessment of chemical quality was undertaken once in six months.

**Table 4: Yield performance of *K. galanga* in open area and under coconut**

K. galanga	Yield at one year (g/plant)		Yield at one year(kg/ha)		Yield at two years(g/plant)		Yield at two years(kg/ha)	
	Open coconut	Under coconut	Open coconut	Under coconut	Open coconut	Under coconut	Open coconut	Under coconut
Dried rhizome	39.2 <sup>a</sup>	45.2 <sup>b</sup>	1410 <sup>a</sup>	1083 <sup>b</sup>	62.7 <sup>b</sup>	76.6 <sup>a</sup>	2253 <sup>a</sup>	1839 <sup>b</sup>

<sup>a</sup> Area outside 2m radius of coconut manure circle. The yield is based on total coconut area and not the effective planted area.

Between columns, values sharing a common letter (super script) do not differ significantly at  $p=0.05$

*Evaluation of the performance of K. galanga in different soil types:* This too was carried out at Walpita estate and the status of the coconut estate was similar to that described in the previous experiment. Three types of soil namely sandy loam, moderately deep gravel and shallow gravel were selected. Experiment was carried out as mentioned before. Five plants were uprooted from each plot once in six months for the dry weight measurement and chemical analysis.

**Table 5: Yield performance of *K. galanga* after one year under coconut in different soils**

Type of soil	Dry weight (kg/ha)	Essential oil content (%)
Sandy loam	849 <sup>a</sup>	5.45
Deep gravel	1317 <sup>b</sup>	3.35
Shallow gravel	1438 <sup>b</sup>	4.36

<sup>a</sup> The values sharing a common letter (Superscript) do not differ significantly at  $p=0.05$

**Table 6: Variation in volatile oil content of rhizome oil of *K. galanga* with maturity and under different conditions**

Age of the plant in years	Yield of volatile oil (%)	
	Under coconut	Open
1	7.85	7.19
1 1/2	3.32	3.08
2	3.31	3.25

**Table 7: Antibacterial activity of *Kaempheria galanga***

Sample	Inhibition zones in cm		
	<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
Rhizome oil 5mg	1.33±0.057	1.2	1.1
Rhizome oil 8mg	1.33±0.04	1.3±0.01	1.00
Root oil 5mg	1.36±0.04	1.2	1.00
Root oil 8mg	1.46±0.044	1.33±0.057	1.00
Gentamicin 5mg	1.43±0.044	1.3	1.33±0.057
Gentamicin 8mg	2.9±0.13	1.9±0.17	2.57±0.115
Control (pentanehexane)	1.00	1.0	1.00

**Antibacterial screening:** The antibacterial activity was studied by the 'Disk diffusion method'<sup>7</sup> using *Staphylococcus aureus* (NCTC 6511), *Escherichia coli*, (NCTC 10418) and *Pseudomonas aeruginosa* (NCTC 10882). The experiment was conducted in triplicate. The MIC values were determined using the serial dilution technique.<sup>8</sup>

**Table 8: The MIC values for *Kaempheria galanga* volatile oils**

Sample	MIC value	
	<i>S. aureus</i>	<i>E. coli</i>
Rhizome oil	40.0mg/ml	80.0mg/ml
Root oil	1.6mg/ml	8.0mg/ml
Gentamicin	1µg/ml	8µg/ml

**Data analysis:** Differences between 'open' and 'under coconut' group were tested for significance using analysis of variance (ANOVA) and chi squared test. Results of antibacterial studies are expressed as  $\pm$ SEM

## RESULTS

Camphene, 1,8 cineol, camphor, borneol, cinnamaldehyde, ethyl cinnamate, quinazoline-4-phenyl-3-oxide and ethyl *p*-methoxycinnamate were detected in rhizome and root oils.

## DISCUSSION

The GLC studies of *K. galanga* indicate that the constituents of rhizome oil and root oil are similar and they differ only in their quantities. In the rhizome oil the content of ethyl cinnamate increased with maturity while that of ethyl *p*-methoxycinnamate decreased. In the root oil the concentrations of both compounds increased with maturity. (Tables 2 and 3) Quinazoline - 4 - phenyl -3- oxide, previously unreported in *K. galanga* essential oils was found to be present. The volatile oil content of rhizomes was higher than that of roots. The yield of rhizome oil decreased with maturity while that of root increased. (Table 1) Though the dry weights of rhizomes increased with maturity (Table 4) the quality decreased as the essential oil content dropped. (Table 6) Therefore it can be concluded that the best time to harvest the rhizomes is at the age of one year. Higher yields were obtained from plants grown in shallow gravel soil compared to those in deep gravel and sandy loam soils (Table 5).

The root and rhizome oils of *K. galanga* showed marked activity against *S. aureus* and *E. coli*. The root oil showed higher activity than the rhizome oil and

this was particularly evident from the more sensitive liquid medium inhibition test. The use of *K. galanga* in traditional medicine may be attributed to these properties.

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