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Research Article

Phytochemical and Nutritional Analysis of Minirhizomes and Mother Rhizomes of a Zingiberaceous Herb *Kaempferia galanga* L.

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ABSTRACT
Kaempferia galanga L. (Family Zingiberaceae), the targeted species of the present study is a high demanding medicinal plant by pharmaceutical companies and in perfume industry. Evaluation of the bioactive constituents would be beneficial in using tissue cultured plants as an alternative for natural plants and the study described here have evaluated the phytochemical parameters of minirhizomes established from micropropagated plants and the mother rhizomes. The phytochemical screening in methanolic extracts of mother rhizomes and minirhizomes of *K. galanga* revealed the presence bioactive substances having a wide range of pharmacological activities such as alkaloids, flavanoids, glycosides, phenols, tannins, saponins and terpenoids in both samples. FTIR analysis of samples showed the presence of alcohols, carboxylic acid, esters, ethers, alkyl halides, aromatic and aliphatic amines and alkenes. The peaks recorded were almost similar in the absorption frequencies in both which confers that the minirhizomes can be used as alternate source materials for drug preparation and essential oil extraction. The indication of esters also confirms the presence of the most significant bioactive compound ethyl p-methoxy cinnamate, which belongs to the class of organic compounds known as cinnamic acid esters in the samples. In TLC analysis, similar type of bands were observed in mother and minirhizomes extracts. The R_f value of the components (R_f = 0.43) is found to be same to that of ethyl p-methoxy cinnamate. The proximate parameters mentioned for both rhizome samples in this study are found to be superior to some other Zingibers. The findings revealed that the minirhizomes can also be explored for phyto-pharma applications. Also, it is a good source of essential nutrients and it could be recommended as a good spice for human diet.

Keywords: minirhizomes, mother rhizomes, *Kaempferia galanga*, proximate analysis, anti-nutritional factors, FTIR, TLC, ethyl p-methoxy cinnamate

INTRODUCTION

The targeted plant material for the present study is *Kaempferia galanga* L. It is commonly known as 'kacholam' (in Malayalam), chandramulika (in Sanskrit), and 'aromatic ginger ' or 'sand ginger 'or ' resurrection lily. The genus received its name from Engelbert Kaempfer, a German botanist. It is a small monocotyledons rhizomatous plant native to India and believed to be originated from Burma. It is mostly cultivated in South East Asian countries such as China, Malaysia, Thailand, Indonesia and India. The plant is famous for its medicinal as well as edible use and found throughout the plains of India under slightly shaded places such as open forest, forest edges and bamboo forest and on various soils. It has been over exploited and listed under threatened category in Sri Lanka and India and in many other Asian countries[1]. *Kaempferia galanga* is a remedy for cough, asthma, fever, swelling and rheumatism. Leaves, rhizome and tubers are the useful plant parts. Commercially rhizomes are used in Ayurveda, Sidha and Folk Medicine for health benefit, food and nutritional purposes. Leaves are used for the treatment of ophthalmia, pharyngodynia, swelling, fever and rheumatism[2]. The species have antioxidant, cytotoxic, anti-inflammatory, sedative, vaso-relaxant, antiangiogenic, anti-nociceptive and wound healing activities[3]. In Thailand, *K. galanga* is used for menstrual disorder and dyspepsia. Traditionally the rhizomes have also been used to treat fever, amoebiasis, dandruff, headache, toothache, rheumatism, abdominal pain, cold and chest pain. It is also a reputed remedy for all diseases caused by the morbidity of vatha and kapha and is useful in respiratory ailments such as cough, bronchitis and asthma. The drug is reported to be acrid, hot, bitter and aromatic and cures skin diseases, wounds and splenic disorders[4]. The rhizome of *Kaempferia galanga* is credited with stimulant, expectorant, diuretic, anti-inflammatory and carminative properties[5]. Powdered

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rhizome is mixed with honey and is given in cough and pectoral affections[6]. Rhizome in the form of powder or ointment is applied on the wounds and bruises to reduce swellings[7]. Roasted rhizome is applied hot in rheumatism and for hastening the ripening of inflammatory tumours. Also rhizomes are used to remove bad odour of the mouth. It is used for protecting cloths against insects and is also used as a deodorant and disinfectant. It is eaten along with betel and areca nut as a masticatory. Also the rhizomes are attached to necklaces for their perfumes. In addition to its traditional uses, experimental studies have demonstrated that *K. galanga* have antioxidant[8], cytotoxic[9], anti-inflammatory[10], sedative[11], vaso-relaxant[12], anti-angeogenic[13], antinociceptive[14] and wound healing activities[15]. These pharmacological activities are due to the presence of different secondary metabolites, among which ethyl p-methoxy cinnamate is the key bioactive secondary metabolite present in the rhizome[16, 17].

The conventional mode of propagation of *K. galanga* is through rhizomes. Since the rhizomes multiplication is slow, the routine propagation of important zingibers was replaced with plant tissue culture-based technologies. The demand of *K. galanga* rhizomes, the commercial part of importance has increased to the extent that the conventional cultivation practices are unable to supply large quantities, hence rapid micropropagation methods are required to meet the target. In zingiber members improved yield and other superior characters have been noticed in *in vitro* plant of *Curcuma* varieties against conventional plants[18]. The microrhizomes are rhizomes produced under *in vitro* condition, while the minirhizomes are the progeny rhizomes produced by the micropropagated plants under *ex vitro* conditions. In addition to these, there are mother rhizomes, which are the seed rhizomes and there exist physiological and environmental differences among the three. However information on the assessment of the quality of minirhizomes against the conventional mother rhizomes are scanty in Zingiberaceae and particularly in *Kaempferia* spp. Thus, comparison of phytochemicals present in rhizomes of natural plants and tissue cultured plants are necessary if the tissue cultured plants are using as alternative to natural plants. The perusal of the literature reveals that the studies regarding the field performance of tissue culture derived plants in terms of phytochemical analysis are not there about *K. galanga* and there are only very little reports with regard to Zingibers[1, 19]. So the present study was carried out to analyse the phytoconstituents of *in vivo* mother rhizomes and minirhizomes raised from micropropagated plants of *K. galanga.*

MATERIALS AND METHODS

Plant material: Rhizomes of *Kaempferia galanga* L. collected from the home cultivation site in Kundara, Kollam District was used as the plant material for the *in vitro* shoot culture establishment and subsequent field transfer experiments in the present study.

In vitro shoot culture establishment

In vitro shoot cultures of *K. galanga* were established from fresh rhizomes with axillary buds collected from the field-grown plants were thoroughly washed under running tap water, to remove the mud and soil particles, outer scales were removed and washed in 5% Teepol (v/v), for 20 minutes and again washed in running tap water. Thereafter several rinses in distilled water, the explants were subjected to sterilization with 0.1% (w/v) mercuric chloride for 8-10 minutes followed by 4-5 rinses in sterile distilled water. Rhizome with axillary buds were inoculated aseptically in Murashige and Skoog (MS) medium[20] containing 0.5 mgl^{-1} Benzyl adenine (BA). As per the established protocol, the initiated shoots were subcultured after 4 weeks to fresh MS medium augmented with 0.1 mgl^{-1} BA and 2.0 mgl^{-1} α-naphthaleneacetic acid (NAA) for shoot multiplication. All the inoculated culture tubes were incubated in a culture room (26±2 ^{o}C) under 8 hour photoperiod at a photon flux intensity of 50-60 $\mu Em^{-2}s^{-1}$ provided by cool, white, fluorescent tubes (Philips, India) under 50-60% RH for eight weeks.

Minirhizome Induction

For minirhizome production *in vitro* plantlets were produced from the previously established in *in vitro* shoot cultures of *K. galanga.* The *in vitro* developed plantlets were deflasked from the culture vessels and were thoroughly washed under running tap water to remove the hormonal milieau and other remnants of culture medium. They were planted in polythene bags filled with garden soil and river sand (3:1), covered with polythene bags and maintained in the green house for 4-6 weeks under regular watering to maintain humidity. The polythene bags were removed gradually after 1-2 weeks period to expose the plantlets to the outer external environmental conditions. The minirhizomes were collected after 6 months for phytochemical analysis.

Phytochemical Analysis

Collection of samples

Healthy *in vivo* rhizomes or mother rhizomes of *K. galanga* collected from the home cultivation sites of Kundara, Kollam District, Kerala, India and minirhizomes collected from the polythene bags from the Department green house were washed well under running

tap water, cleaned, weighed and dried at ambient temperature. The dried rhizomes were again weighed and powdered. Samples were kept in airtight containers for the phytochemical analysis.

Preparation of extracts

The phytochemicals present in the rhizome powder samples were extracted by the distillation method using Soxhlet apparatus. Methanol was used as the solvent for the extraction of chemicals in *in vivo* mother rhizomes and minirhozomes of *K. galanga*. About 20 g powdered sample was weighed, packed and kept in the soxhlet apparatus. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at the boiling point of methanol. The extract was concentrated to dryness and the residue were transferred to pre-weighed sample bottles and were stored in desiccator for further studies.

Qualitative analysis

Preliminary phytochemical tests were performed with the rhizome samples for the detection of secondary metabolites like reducing sugars (Fehling's test), flavanoids (Shinoda test), terpenoids and steroids (Liebermann-Burchard test), tannins, coumarins, alkaloids, saponins, anthraquinones, glycosides (Keller-Killiani test), phlobatannins, iridoids, phenols ($FeCl_3$ test) and amino acids (Ninhydrin test) using standardized procedures[21].

FTIR profiling

For IR profiling, fresh *in vivo* mother rhizomes and minirhizomes of *K. galanga* collected were dried in an oven (Labline, India) for 2 days at 60 ºC. Tablets for FTIR spectroscopy were prepared in an agate mortars by mixing the sample powder with (2 mg) with KBR (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}. At least three spectra were obtained for each sample.

A FTIR spectrometer (FTIR Schimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0 cm^{-1}. De-convolved and second derivative spectra were calculated for Fourier self-deconvoltion, the bands were selected and normalized to unity with Omnic 7 software. Curve fitting of the original spectra was performed with Origin 7 software. The band position of functional group was monitored with knowitall 7.8 software. The spectral region between 3000 and 2800 cm^{-1} was selected to analyze lipids and that between 1800 and 1500 cm^{-1} was selected to analyze proteins. The spectral region between 1200 and 1000 cm^{-1} was selected to analyze carbohydrates. Triplicate experiments were conducted and spectra from the first two times of experiments were used for establishment of chemometric models and the spectra from the third time of experiment was used for model validation.

Proximate analysis

The proximate composition such as moisture, ash and fibre content were estimated in the rhizome samples of *K. galanga*. Total carbohydrates and protein contents were also calculated [22, 23]. The methodologies described below.

a. Moisture content

For determination of moisture, accurately weighed (5g) quantity of the mother rhizomes and minirhizomes were heated in a hot air oven at 105 °C for 2 hours and cooled in desiccator, again weighed and moisture content was calculated by the following formula,

$$\textbf{Moisture}\ (\%) = \frac{(\text{Fresh Wt of sample} - \text{Dry Wt}) \times 100}{\text{Fresh Weight of sample}}$$

b. Ash content

For ash content estimation, 1g sample material placed in crucible was combusted in muffle furnace at 500 ºC. The percentage of ash content was calculated using the formula

$$\textbf{Ash}\ (\%) = \frac{\text{Weight of ash (g)} \times 100}{\text{Weight of original sample (g)}}$$

c. Crude fibre content

A weighed quantity of dried sample was digested using 250 ml of 0.12 NH_2SO_4 for half an hour and filtered. The residue was washed several times with distilled water until all the acid soluble components were removed. The residue was then boiled with 250 ml of 0.313 N NaOH for half an hour to remove alkali soluble components. After filtration, the residues were dried, weighed and ignited in a muffle furnace. The percentage of crude fibre was calculated as:

$$\textbf{Crude fibre}\ (\%) = \frac{\text{Weight loss on ignition (g)} \times 100}{\text{Weight of original sample (g)}}$$

Determination of anti-nutritional factors

Quantitative estimation of anti-nutritional factors like total phenolics[24], saponins, tannins[25] and phytic acid[26] were carried out in mother rhizomes and minirhzomes of *K. galanga* as mentioned below.

Thin Layer Chromatography (TLC)

Methanolic extract of mother rhizome and minirhizomes of *K. galanga* were used for TLC analysis and were spotted on precoated silica gel on aluminium sheets (TLC silica gel 60 F224) (Sigma Aldrich). Here, hexane ethyl acetate (9:1) was used as the solvent system and silica gel was used as the stationary phase. After running the samples, the spots formed were visualized in a UV-chamber (Kemi).

To calculate the R_f value, the distance travelled by the substance being considered is divided by the total distance travelled by the mobile phase.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

RESULTS AND DISCUSSION

Phytochemical Analysis

Phytochemicals are bioactive nutrient chemicals found in vegetables, fruits, grains and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases[27]. These are primarily 'secondary metabolites' which show highly bioactive nature and they include alkaloids, tannins, flavanoids, saponins, glycosides, phytates and coumarins. The present study comprised phytochemical analysis in terms of qualitative and quantitative parameters in the mother rhizomes and minirhizomes of *K. galanga* and the findings are detailed below.

Qualitative analysis of phytochemicals

The preliminary phytochemical screening of qualitative analysis in methanolic extracts of mother rhizomes and minirhizomes of *K. galanga* revealed the presence of alkaloids, flavanoids, glycosides, phenols, tannins, saponins and terpenoids in both. However, amino acids, coumarins, anthraquinones, phlobatannins and iridois were absent in both the extracts (Table 1). All these substance have wide range of pharmacological activities. Many phytoconstituents have antioxidant activity that protect the cells against oxidative damage and reduce the risk of diseases. Flavanoids are widely distributed in plants fulfilling several functions and they enhance the effect of Vitamin C function as antioxidants[28]. Alkaloids and saponins have been reported to possess antibacterial activities[29]. Saponins also have been reported to show antimicrobial properties[30] and they act as an important precursor for steroidal substances. The terpenoids exhibit anti-inflammatory and antimicrobial effect[31]. Glycosides are the drugs used in the treatment of congestive heart failure and cardiac arrhythmia[32]. Tannins have antiviral, antibacterial and antiparasitic effects[33].

Table 1: Preliminary phytochemical analysis of mini and mother rhizomes of *K. galanga*

Sl. No	Phytochemical Constituents	Test	Inference of phyto-constituents	Methanol extract	
				Mother rhizome	Mini rhizome
1	Reducing sugar	Fehling's Test	Formation of a brick red colour	+	+
2	Flavanoids	Shinoda test	A pink, scarlet, crimson red or occasionally green to blue colour appeared after few minutes	+	+
3	Terpenoids	Liebermann – Burchard Test	Appearance of pink colour	+	+
4	Tannins	Lead acetate test	A red brown color formed at the interface	+	+
5	Coumarins	NaOH-HCl test	The appearance and disappearance of yellow colour	-	-
6	Alkaloids	Dragendroff reagent	Formation of a reddish brown precipitate	+	+
7	Saponins	Froth test	Stable foam	+	+
8	Anthraquinones	Test for anthraquinone	Presence of delicate rose pink colour	-	-
9	Glycosides	Keller-Killani's test	A brown ring at the interface	+	+
10	Phlobatannins	HCl test	Formation of red precipitate	-	-
11	Iridoids	Test using acetic acid and $CuSO_4$	Development of light blue colour	-	-
12	Phenols	$FeCl_3$ test	Formation of greenish black colour	+	+
13	Amino acids	Ninhydrin Test	Development of a pink purple or violet blue colour	-	-

+ Present; - Absent

Phytochemical screening by Infra-Red Spectroscopy

The FTIR spectrum was mainly used for identifying the functional group of the active compounds based on the peak value in the region of infrared radiation. It is an analytical technique which uses infrared light to identify various functional groups in unknown substances through the identification of different covalent bonds that are present in the compound. The wavelength of light absorbed is characteristics of the chemical bond and can be seen in the annotated spectrum. This technique promise to be great value because of its simplicity, rapidity, sensitivity and low expense. One of the

important application of the infrared spectroscopic study is its diagnostic value in establishing the presence of certain organic constituents in plants[34]. Here in this study, dried powder of the minirhizomes and mother rhizomes of *K. galanga* were the samples used for FTIR analysis. When the rhizome powder was passed into the FT-IR, the functional groups of the components separated based on its peak ratio (Figs. 1a & 1b).

a. FTIR analysis of mother rhizomes

In the FTIR spectrum of mother rhizomes of *K. galanga*, 18 major peaks were identified (Table 2). Alkanes, alkenes, amines, alkyl halides, nitro compounds, carboxylic acids, esters and ethers were the important functional groups noticed. The highest wave number 2924.46 cm^{-1} contains C-H stretch with alkanes and 3275.57cm^{-1} indicated the presence of terminal alkynes in –C (triple bond) C-H: C-H stretch. The peak value at 1604.31 cm^{-1} and 1633.70 cm^{-1} designated the presence of primary amines and N-H bend. The peaks around 1000-1500cm^{-1} represent C-O stretch which specified alcohols, carboxylic acids, esters, ethers, C-N,=CH,O-H,N-O (asymmetric stretch), C-H Wag(-CH_2 X), C-C (in ring) and nitro compounds (N-O asymmetric stretch). The area of peaks in between 560-850 cm^{-1} (569.37, 764.97, 827.68 cm^{-1}) represented alkyl halides (C-Cl stretch).

Table 2: IR Frequency table of mother rhizomes of *K. galanga*

Sl. No.	Frequency	Bond	Functional group
1	521.46	CH bend	Alkenes
2	569.37	C-Cl stretch	Alkyl halides
3	764.97	C-Cl stretch	Alkyl halides
4	827.68	C-Cl stretch	Alkyl halides
5	927.04	O-H bend	Carboxylic acid
6	994.04	=C-H bend	Alkenes
7	1075.09	C-O stretch	Aliphatic amines
8	1154.37	C-H wag(-CH2X)	Alkyl halides
9	1203.40	C-N stretch	Aliphatic amines
10	1251.27	C-N stretch	Aromatic amines
11	1311.38	C-O stretch	Alcohols, carboxylic acid, esters, ethers
12	1366.44	C-H rock	Alkanes
13	1420.98	C-C stretch	Aromatics
14	1512.92	N-O asymmetric stretch	Nitro compounds
15	1604.31	N-H bend	Primary amines
16	1633.70	N-H bend	Primary amines
17	2924.46	C-H stretch	Alkanes
18	3275.57	C (triple bond) C-H:C-H stretch	Alkynes (terminal)

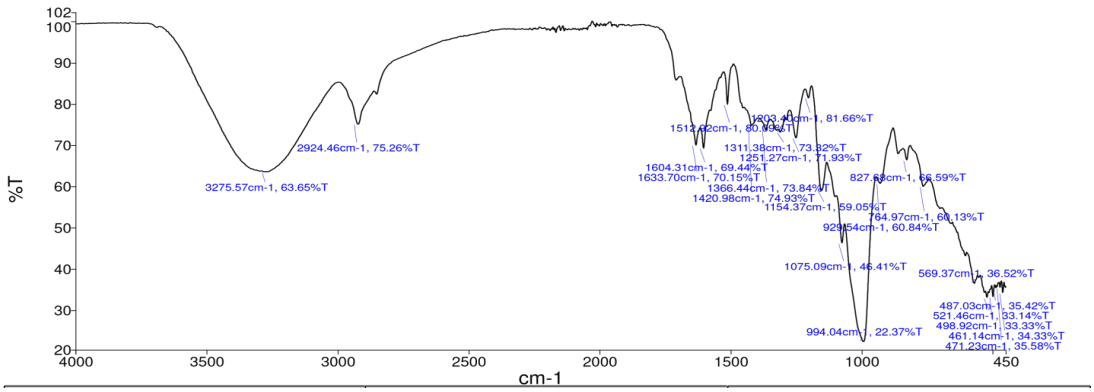

Figure 1a: IR Spectral diagram of mother rhizome of *K. galanga*

b. FTIR analysis of minirhizomes

FTIR analysis in minirhizomes of *K. galanga* depicted similar absorption frequencies of mother rhizome samples (Figs. 1a&b) and confirmed the presence of C-H, N-H, C-N, C-O, C- Br and C-H wag (-CH_2X) functional groups. From this FTIR spectrum, the spectra of wave numbers in between 500 cm^{-1} and 4000 cm^{-1}, ten major peaks were identified (Table 3). The highest frequency was 3276.06 cm^{-1}, that indicated N-H stretch and primary secondary amines and amides. A particular major reflection peak

identified in the band 1074.81cm^{-1}. Aliphatic amines are present at the peak value 1074.81 cm^{-1} contain C-N stretch. The peak around 1250-1650 cm^{-1} denoted amines while 2850-3000 cm^{-1} specified alkanes (C-H Stretch). The peak value 1150.31 cm^{-1} indicated the presence of alkyl halides with C-H Wag (-CH2X). This peak indicated the presence of C-N Stretch (aliphatic amines). The peak 518.26 shows C- Br stretch bond and it referred to the presence of functional group alkyl halides. Alcohols, carboxylic acids, esters, ethers are seen in C-O stretch at the peak value 1002.71 cm^{-1}.

Table 3: IR Frequency table of minirhizomes of *K. galanga*

Sl. No.	Frequency	Bond	Functional group
1	518.26	C- Br stretch	Alkyl halides
2	1002.71	C-O stretch	Alcohols, carboxylic acid, esters, ether
3	1074.81	C-N stretch	Aliphatic amines
4	1150.31	C- H wag(-CH2X)	Alkyl halides
5	1249.38	C--N stretch	Aliphatic amines
6	1321.67	C-N stretch	Aromatic amines
7	1635.46	N -H bend	Primary amines
8	2852.07	C H stretch	Alkanes
9	2921.54	C-H stretch	Alkanes
10	3276.06	N-H stretch	Primary, secondary amines, amides

Thus the phytochemical screening of chemical constituents of *K. galanga* rhizome powder samples of mother rhizome and mini rhizome using IR spectroscopy showed the presence of alcohols, carboxylic acid, esters, ethers, alkyl halides, aromatic and aliphatic amines, alkenes, etc. The peaks recorded were almost similar in the absorption frequencies in both rhizome samples also (Figs. 3a & b) which confers that the minirhizomes can be used as alternate source materials for drug preparation and essential oil extraction. The indication of esters also confirms the presence of the most significant bioactive compound ethyl para-methoxy cinnamate, which belongs to the class of organic compounds known as cinnamic acid esters in both rhizome samples of *K. galanga*.

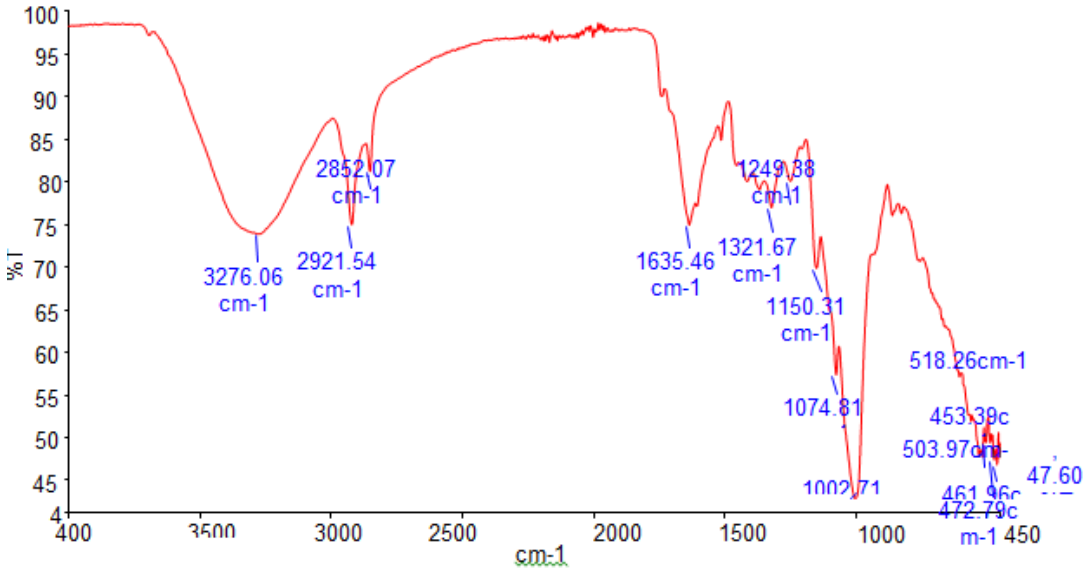

Figure1b: IR spectral diagram of minirhizomes of *K. galanga*

Proximate Analysis

Important nutritional aspects of mother rhizomes and minirhizomes of *K. galanga* has been listed in Table 4. Ash content analysis and moisture content analysis are the two important parameters which may directly affects the nutritional parameters of *K. galanga* rhizome[35]. Ash contents of mother rhizomes and minirhizomes of *K. galanga* were approximately equal (57.15±0.01 and 58.10± 0.04 mgg^{-1} respectively). In this study, moisture content of mother rhizomes and minirhizomes of *K. galanga* were also alike. This parameter may be comparatively lower in the dried samples which offers its usefulness for storage purposes and it is also helpful to increase the shelf-life of this rhizomatous species.

The amount of carbohydrate in minirhizomes was relatively higher than that of mother rhizomes. i.e., the amount of carbohydrates in mother rhizome was 200.23 mgg^{-1} and the same in minirhizomes was 208.54 mgg^{-1} and were found to be high. This is comparable with the carbohydrate contents of some important cereals[36] and higher than other Zingiberaceae species[37]. In the case of crude fibre, more fibre content was recorded in minirhizomes (65.99 mgg^{-1}) and a good amount of fibre was present in mother rhizomes i.e., 61.82 mgg^{-1}. Appreciable amount of protein contents were estimated in both *in vivo* mother rhizomes and minirhizomes wherein the minirhizomes contain more amount of protein (48.78 mgg^{-1}) than that of mother rhizomes (45.23 mgg^{-1}) (Table 4). The proximate parameters mentioned for both rhizome samples in this study are found to be superior to some other reported Zingiberaceae rhizomes[37]. Thus the proximate composition of *K. galanga* rhizome samples revealed that the minirhizomes can also be explored for phyto-pharma applications. Also, it is a good source of essential nutrients and it could be recommended as a good spice for human diet.

Table 4: Proximate compositions in fresh samples of mini and mother rhizomes of *K. galanga*

Type of Biochemical	Quantity (mgg^{-1})	
	Mother rhizomes	Minirhizomes
Ash content	57.15 ± 0.01^a	58.10 ± 0.04^a
Moisture Content	70.38 ± 0.03^a	68.17 ± 0.02^b
Crude fibre	61.82 ± 0.04^b	65.99 ± 0.03^a
Total Carbohydrate	200.23 ± 0.02^b	208.54 ± 0.4^a
Total Protein	45.23 ± 0.03^b	48.78 ± 0.03^a

*Data represents mean values of six replicates repeated thrice. The mean values followed by the same letter in the superscript in a row do not differ significantly based on ANOVA and t-test at $p\leq0.01$.

Determination of anti-nutritional factors

'Antinutritional factors' are the substances or chemical compounds found in fruits and food substances which are poisonous to human or in some way, limit the nutrients' availability to the body. These factors are known to interfere with metabolic process such that growth and bioactivity of nutrients are negatively influenced. They include tannins, oxalates, phytic acid and saponins whose presence greatly impair the digestion of various nutrients, thereby reducing the nutritional value of plants and limiting their utilization as food[38]. Phytic acid binds with calcium, iron, zinc and other minerals thereby reducing their availability in the body. It also inhibit protein digestion by forming complexes with them and decreases calcium availability by forming calcium phytate complexes that inhibit the absorption of Fe and Zn[39]. Oxalate and its contents have deleterious effect on human nutrition and health, mainly by decreasing calcium absorption and serve the formation of kidney stones[40]. Tannins are known to inhibit the activities of digestive enzymes and hence the presence of even low level of tannin is not desirable from nutritional point of view[41].

Saponins are another antinutritional substances that reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intra-lumenal physio-chemical interaction. Hence saponins has been reported to have advantageous hypo cholesterolemic effects[42].

Antinutritional factors present in mother rhizomes and minirhizomes of *K. galanga* were analysed in the present study and are summarized in Figs. 2. Phenols, tannins, phytic acids and saponins were the main antinutritional components present in both samples. The concentration of total phenol in mother rhizome was 0.80 mgg^{-1} while it was 0.85 mgg^{-1} in minirhizomes. The amount of tannins present in mother rhizomes was 0.10 mgg^{-1} and it was slightly increased to 0.11 mgg^{-1} in minirhizomes of *K. galanga.* The phytic acid content in mother rhizomes was 0.46 mgg^{-1} and it was reduced to 0.44 mgg^{-1} in minirhizomes. The concentration of phytic acid content in mother and minirhizomes were less than that of major cereals and other ginger species[43]. The amount of saponins in the case of mother rhizome was 0.32 mgg^{-1} and it was 0.31 mgg^{-1} in minirhizhomes.

Among these different anti-nutritional components, amount of phenol was high as they are one of the largest and most ubiquitous groups of plant metabolites. Some phenols are proved to have hypotensive effect and antioxidant properties[44]. Hence the high phenolic concentration can offer antioxidant property to mother and minirhizomes of *K. galanga*. Most of the antinutritional factors play an important role in plant defense mechanism. All these anti-nutritional factors were present in minute quantities which highlight the nutritional effects of mother and minirhizomes of *K. galanga.*

The quantitative phytochemical evaluation of rhizome samples (mini and mother rhizomes) revealed good proximate composition and acceptable anti-nutritional levels which demonstrated that this culinary spice has all

the essential requirements to be developed as a phyto-nutraceutical resource. Since the minirhizome exhibited superior qualities than the mother rhizomes in majority of the parameters tested, it can also be concluded that the minirhizomes raised through plant tissue culture technology can be utilized for pharma needs with more accuracy than the rhizomes obtained through conventional breeding programmes.

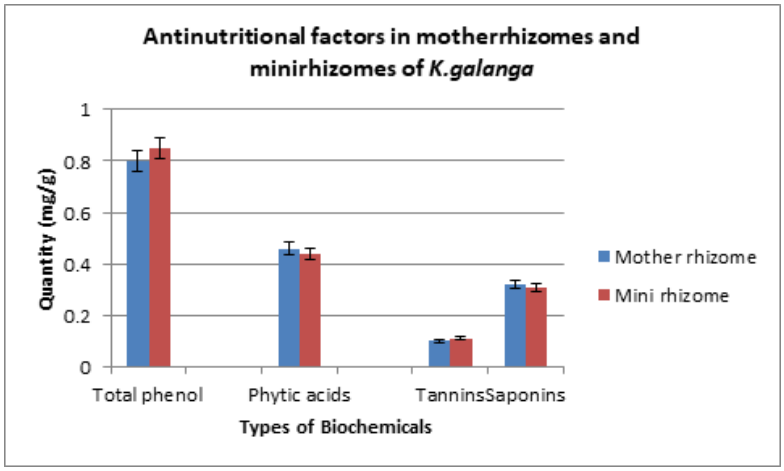

Figure 2: Quantification of anti-nutritional factors in mother and minirhizomes of *K. galanga*

Thin Layer Chromatography

Thin layer chromatography, a technique used to separate non-volatile mixture is performed on a sheet of an aluminium foil which is coated with a thin layer of adsorbent material silica gel. This layer of adsorbent known as stationary phase. After the sample have been applied on the plate, a solvent mixture (mobile phase) is drawn the plate via capillary action. After the experiment the spots were visualized by projecting ultraviolet light on sheet.

In TLC analysis, similar type of bands were observed in mother and minirhizomes extracts of *K. galanga* (Fig. 3). The R_f value of these bands were calculated (R_f = 0.43) and it is found to be same to that of the R_f value of ethyl p- methoxy cinnamate based on previous report (https://www.globinmed.com Malasian Herbal Monograph)

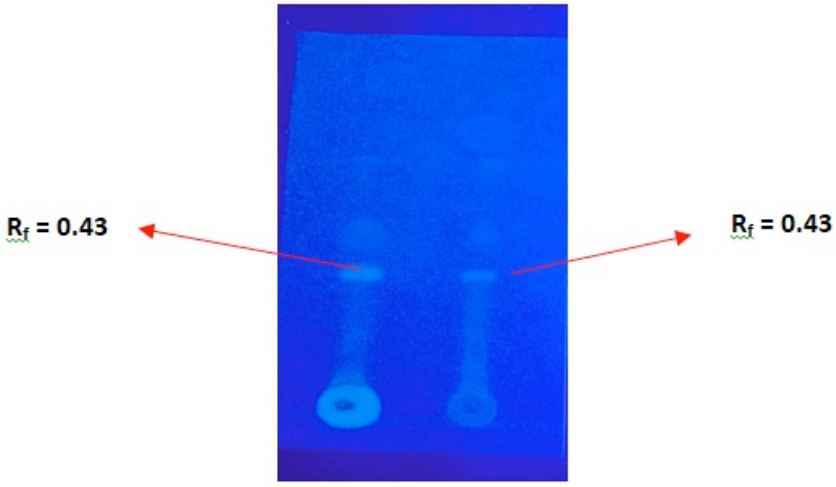

Figure 3: *K. galanga* rhizome samples in TLC [Left– Mother rhizome; Right– Minirhizome]

CONCLUSION

The quantitative phytochemical evaluation of rhizome samples (mini and mother rhizomes) revealed good proximate composition and acceptable anti-nutritional levels which demonstrated that this culinary spice has all the essential requirements to be developed as a phyto-nutraceutical resource. Since the minirhizome exhibited superior qualities than the mother rhizomes, the minirhizomes can also be utilized for pharma needs with more accuracy than the rhizomes obtained through conventional breeding programmes.

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Conflicts of interest

There is no conflict of interest.

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