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Research Article

Metabolic profiling of *Kaempferia galanga* leaf and rhizome extract using GC-MS

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ABSTRACT

Kaempferia galanga Linn. is an endangered rhizomatous medicinal plant belonging to the Zingiberaceae family. It has evolved as an emerging industrial crop and dominates pharma as well as aroma sector. Though the extracts of this species have been extensively used in herbal medicine across the globe for the treatment of numerous diseases, but still the composition of the extract is not characterized properly. Thus, methanol extracts of *K. galanga* leaves and rhizomes were subjected to phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the phytoconstituents. Leaf extract contained more TPC and TFC values as compared to rhizome extract. A total of eight and ten compounds were identified in the leaf and rhizome extract accounting for 61.44% and 96.97% of the total peak area respectively. Ethyl p-methoxycinnamate was found as the main constituent in rhizome extract covering 80.39% of the total area. Other important compounds like ethyl cinnamate (9.61±0.45%), pentadecane (3.12±0.2%) were also found in the rhizome extract, whereas leaf extract contained 2-(3,4-dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-chromen-4-one (18.26%), 2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one (14.01%), octamethylcyclotetrasiloxane (11.79%). The study indicated that *K. galanga* is a good source of phytoconstituents which can be used at the industrial level to produce pharmaceuticals, perfumes and flavouring agents.

Key words: *Kaempferia galangal*, phytoconstituents, GC-MS, TPC, TFC

INTRODUCTION

Natural plant products in the form of medicines have been used to cure several acute and chronic diseases since time immemorial. A lot of new discoveries and current therapies are actually based on this ancient medicine. Documentation of plants for their ethnopharmacological properties has been accounted for the last 1000 years. Around half of the present-day drugs are synthesized from plant and natural sources (Gurib-Fakim, 2006; Dias *et al.,* 2012).

Plants play a vital part in both development as well as the synthesis of chemotherapeutic drugs because of their bioactive constituents (Tona *et al.,* 1998). Due to the vast biodiversity and less research, a lot of biological and pharmacological properties of plants are not well known. Specifically, in medicinal plants, scientists and researchers around the world are continuously working on exploring the potency of bioactive natural pharmacological constituents present in medicinal plants (Karmegam *et al.,* 2012). Studies also reveal that 80% people of the world's population relies upon herbal medications due to their high potency to fight against diseases, lesser side effects, nonnarcotic and cost-effective nature (Ahmad & Beg, 2001).

Plants produce secondary metabolites such as phenols, terpenes, flavonoids etc. to resist them from various plant pathogens e.g., insects, bacteria, fungus, nematodes, and herbivorous animals. This defence mechanism of plants in the form of secondary metabolites also plays a tremendous role in curing various human diseases. These secondary metabolite constituents are in fact a part of many industrial, therapeutic products, herbal drugs, beverages, nutraceuticals, perfumes etc. (Hadian *et al.,* 2014). Amidst all the natural bioactive constituents, terpenes play a significant role in the treatment of human diseases (Padalia, 2012).

K. galanga Linn. (aromatic ginger) of the family Zingiberaceae is an endangered rhizomatous plant having tremendous medicinal activities. Several industries are in need of its plant rhizome extracts for drug development as well as in the preparation of perfumes (Rahman *et al.,* 2004). Also, *K. galanga* leaves are widely used to prepare mouthwashes, food flavours, hair tonics etc. (Sulaiman *et al.,* 2008). Besides this, rhizomes of *K. galanga* have served as a major relief for skin diseases, asthma, malaria, bronchitis, splenic disorders, etc. (Kochuthressia *et al.,* 2012). The plant is reported to have antimicrobial, antimalarial, larvicidal properties, antioxidant, antinociceptive and anti-inflammatory activities (Hanumantharaju *et al.,* 2010; Sumazian *et al.,* 2010; Thiengsusuk *et al.,* 2013; Satoto *et al.,* 2013). Some current findings also show that *K. galanga* has evolved as an emerging industrial crop and dominates both the pharma as well as aroma sector (Yao *et al.,* 2018). Ethyl p-methoxycinnamate (EPMC), penta decane, methyl cinnamate, etc., are found to

be the major bioactive constituents reported in *K. galanga* essential oil (Tewtrakul *et al.,* 2005; Mohanty *et al.,* 2011). Again, EPMC extracted from *K. galanga* rhizomes was found to be highly cytotoxic to HeLa cells (Mohanty *et al.,* 2011). EPMC possesses anti-inflammatory, antineoplastic, hepatoprotective activities (Manigaunha *et al.,* 2010; Sirisangtragul & Sripanidkulchai, 2011; Umar *et al.,* 2011).

Several chromatography techniques are used to analyse the quality of herbal extracts and for profiling plant secondary metabolites. GC-MS analysis is one of the most appropriate and trustworthy systems broadly used for the identification and determination of volatile phytoconstituents. Various researchers reported the occurrence of diverse phytochemicals in many species using this technique (Sahoo *et al.,* 2014; Sahoo *et al.,* 2017; Sahoo *et al.,* 2020; Dash *et al.,* 2021; Singh *et al.,* 2021). Likewise, the phytoconstituents of the rhizome essential oil of *K. galanga* have been studied by several researchers (Ravindran & Balachandran, 2005; Tewtrakul *et al.,* 2005; Mohanty *et al.,* 2011; Sahoo *et al.,* 2014). However, few reports are available on the chemical composition of the rhizome extract of this species. So far, only two studies have been reported about the chemical composition of methanol extract and the fraction of chloroform extract of *K. galanga* rhizome (Umar *et al.,* 2012; Ali *et al.,* 2018). But there is no report available yet, on GC MS analysis of leaf extract of *K. galanga*. This is the first report on the chemical constituent analysis of *K. galanga* leaf extract.

Taking this problem in the background, this study was carried out to determine various volatile bioactive compounds that are present inside *K. galanga* leaf and rhizome extract with the aid of the GC-MS technique, which will further help to figure out its use in traditional medicine. The present study is also aimed at phytochemical screening, total phenolic and also flavonoid content of leaves and rhizomes of *K. galanga* for the extensive analysis of its phytoconstituents.

MATERIALS AND METHODS

Plant material and extract preparation

Rhizomes of *K. galanga* were brought from the Silviculture department, Odisha and then it was identified by a taxonomist of the Regional Plant Resource Centre (RPRC), Bhubaneswar. After successful identification of the plant rhizome, it was then planted inside the medicinal plant house of the Centre for Biotechnology, Siksha 'O' Anusandhan deemed to be University, Bhubaneswar. Again, after the complete growth of the plant, fresh leaves, as well as rhizomes, were collected, properly washed, and chopped thoroughly into pieces followed by shade drying for at least 15-20 days. Then the dried leaves and rhizomes were powdered and extracted separately using extra pure methanol (AR grade 99.8%) in the Soxhlet apparatus for 24 hours. The methanol extracts were then filtered with Whatman 40 filter paper and the solvent was evaporated using a rotary evaporator to yield a semisolid mass (11.5% w/w). The resulting extracts were stored in the refrigerator for future study.

Phytochemical screening

The methanol leaf and rhizome extracts of the *K. galanga* were separately subjected to preliminary phytochemical screening for the identification of different chemical groups (Kokate, 1994; Harborne, 1998).

Evaluation of total phenolic and total flavonoid contents

Total phenolic content (TPC) of both the leaf and rhizome extracts of *K. galanga* was determined by the Folin-Ciocalteu method as described by Sahoo *et al.* (2013) using Gallic acid as a standard. Likewise, the total flavonoid content (TFC) of the extracts was estimated using the aluminium chloride calorimetric method (Sahoo *et al.,* 2013). TFC was then calculated from the calibration curve of Quercetin. TFC was expressed as mg Quercetin equivalent/g of the extract. All the determinations were performed in triplicate.

Gas chromatography-mass spectrometry analysis

GC-MS (gas chromatography-mass spectrometry) analysis was done by using a 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA) attached with a mass selective detector having a quadruple analyzer, MSD 5973. The electron ionisation energy, ion source temperature and interface temperature were 70 eV, 230°C and 280°C respectively. A split-split less injection having a split ratio of 1:100 was employed at 250°C injector temperature. A fused silica column HP-5 (30 m × 0.25 mm i.d and 0.25 µm film thickness) was used in this study. The oven temperature was programmed like this: from 50°C-240°C at 4°C/min; from 240°C to 270°C at 15°C/min; held isothermal at 50°C for 1 min and 270°C for 15 min. Then the essential oil sample of 1µl was injected into the GC. Helium was used as carrier gas at a flow rate of 1ml/min. Further data acquisition was carried out with the software for the mass ranging 50-600amu with a scan speed of 1 scan/sec. The compound identification was done by comparing their mass spectral data with the National Institutes of Standards and Technology (NIST, USA) library. The percentage of occurrence of constituents was obtained by peak area normalization. Response factors were not calculated.

RESULT AND DISCUSSION

Phytochemical screening

For the discovery of novel drugs, phytochemical screening of plant extracts plays a vital role by providing the necessary information in the form of phytochemical constituents. In the current investigation, as revealed from preliminary phytochemical screening, the leaf and rhizome extract of *K. galanga* contained different bioactive phytoconstituents which are given in Table 1. In leaf extract alkaloids, flavonoids,

Table 1: Phytochemical screening of leaf and rhizome extract of *K. galanga.*

Phytoconstituents	Test performed	K.G Leaf	K.G Rhizome
Alkaloids	Dragendroff's test	+ve	+ve
	Mayer's test, Wagner's test, Hager's test	+ve	+ve
Steroids	Libermann Burchard test, Salkowski test	+ve	+ve
Flavonoids	Alkaline reagent test, Shinoda test	+ve	+ve
Triterpenoids	Libermann Burchard test, Salkowski test	-ve	+ve
Carbohydrates	Molisch's test, Fehling's test	-ve	+ve
	Barfoed's test, Benedict's test	-ve	-ve
Tanins	$FeCl_2$ test	+ve	+ve
Saponins	Foam test	+ve	+ve
Aminoacids	Millon's test, Ninhydrin test	-ve	-ve
Glycosides	Killer-Kiliani test	-ve	+ve
	Brontrager's test	-ve	-ve

(+): Indicates the presence of chemical constituents, (-): Indicates the absence of chemical constituents

steroids, saponins and tannins were found positive and carbohydrates, amino acids, triterpenoids, saponins and glycosides were found negative. Similarly, rhizome extract was found to contain alkaloids, flavonoids, steroids, triterpenoids, saponins and tannins. These phytochemicals show certain pharmacological properties. Alkaloids are well known for having antihypertensive and detoxifying activities. Generally, flavonoids inhibit those microbes which remain resistant to antibiotics. Similarly, tannins are also known as antimicrobial agents (Subramanian & Suja, 2011). Antibacterial activities have also been reported from steroids (Epand *et al.,* 2007). The present study, thus reveals the amazing medicinal significance of *K. galanga*, due to the presence of these above-described valuable bioactive natural compounds and also strongly suggests the development of new pharma products from *K. galanga* for benefit of human health.

Evaluation of total phenolic and flavonoid contents

TPC of leaves and rhizomes extract of *K. galanga* was found to be 51.5±0.67 mg GAE/g and 33.5±0.84 mg GAE/g of the extract as calculated from the standard curve of Quercetin. TFC of *K. galanga* leaves and rhizomes extract was found to be 47±0.21 and 39.38±0.75 mg Quercetin equivalent/g of extract.

For many years, various studies and findings on medicinal plants have revealed their mechanism of action which was then claimed by the traditional healers. Among the plant secondary metabolites, phenolics being the largest group shows myriad biological activities such as anti-inflammation, anticarcinogen, antiaging, antiapoptosis, cell proliferation, cardiovascular protection, etc. (Han *et al.,* 2007; Singh *et al.,* 2007). Further, in terms of their capability to work as radical scavengers, these above diverse groups of bioactive compounds serve as potential natural antioxidants.

The phenolics and flavonoids show myriad pharmacological properties which might be related to their antioxidant activity, so it is quite necessary to figure out the TPC and TFC of various plant extracts (Chung *et al.,* 1998; Mathew & Abraham, 2006; Elzaawely *et al.,* 2007). So, therefore we can conclude that the antioxidant capacity of the plants is mainly attributed to their phenolic compounds (Huang *et al.,* 2009).

GC-MS analysis of extracts

Gas chromatography and mass spectrometry analysis were conducted for determining volatile phytochemical constituents present in the leaves and rhizomes extract of *K. galanga*. A total of 8 and 10 components were identified accounting for 61.44% and 96.97% of the leaf and rhizome extract respectively (Fig. 1 and 2). The major constituents of the leaf extract were 2-(3,4-dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-chromen-4-one (18.26±0.35%), 2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one (14.01±0.3%), octamethylcyclotetrasiloxane (11.79±0.2%) whereas rhizomes extract contained ethyl p-methoxycinnamate (80.39±0.85%), ethyl cinnamate (9.61±0.45%), pentadecane (3.12±0.2%) as major constituents (Table 2 and 3). The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Fig. 1.

The chemical profile of methanol extract of *K. galanga* rhizome was done by GC-MS analysis and the major eight components were 2-propenoic acid (10.18%), phthalic acid (3.37%), palmitic acid (35.17%), sandaracopimaradiene (8.20%), oleic acid (22.15%), octadecanoic acid (10.10%), 2-[2-(4-nonylphenoxy) ethoxy] ethanol (3.57%) and glycidyl stearate (7.27%) were found in the methanol extract, but there is an absence of ethyl-p-methoxycinnamate which is the main constituent found in our study (Ali *et al.,* 2018). Ethyl-p-methoxycinnamate (EPMC) was found as the most abundant compound in the sub-fraction of chloroform extract of *K. galanga* rhizome as analyzed by GC-MS (Umar *et al.,* 2012). This is in agreement with our result that EPMC was also the major constituent of methanol extract of *K. galanga* rhizome. EPMC was found as the chief component in the rhizome essential oil of *K. galanga* as revealed by most of the studies (Tewtrakul *et al.,* 2005; Ravindran & Balachandran,

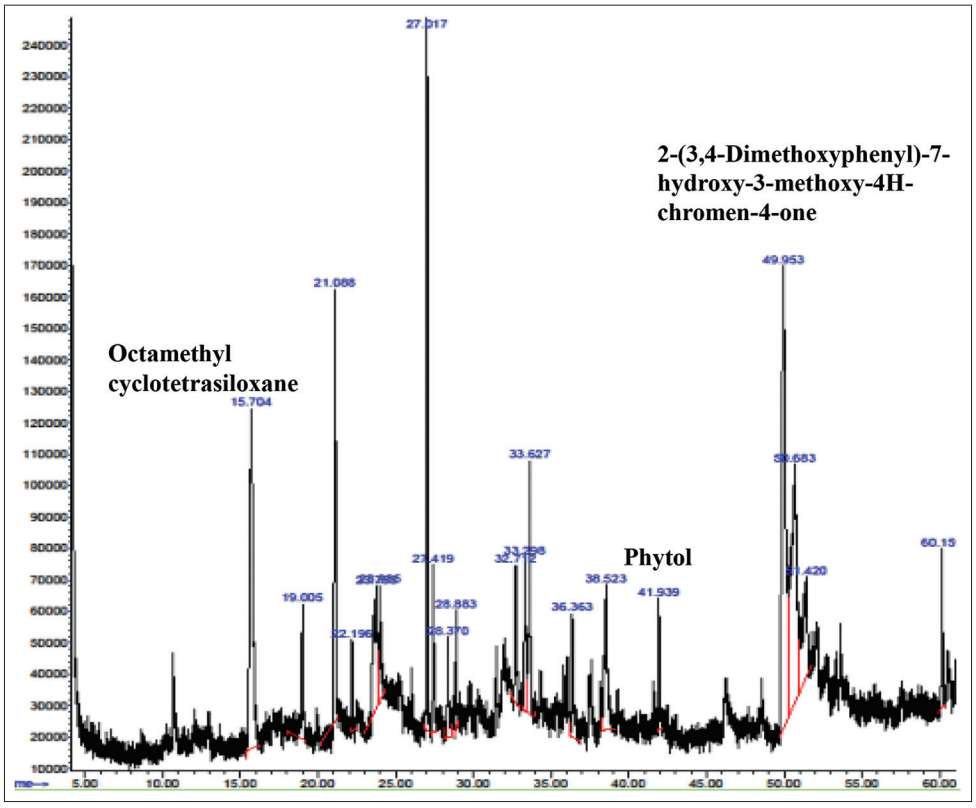

Figure 1: GC-MS Chromatogram of *K. galanga* leaf extract

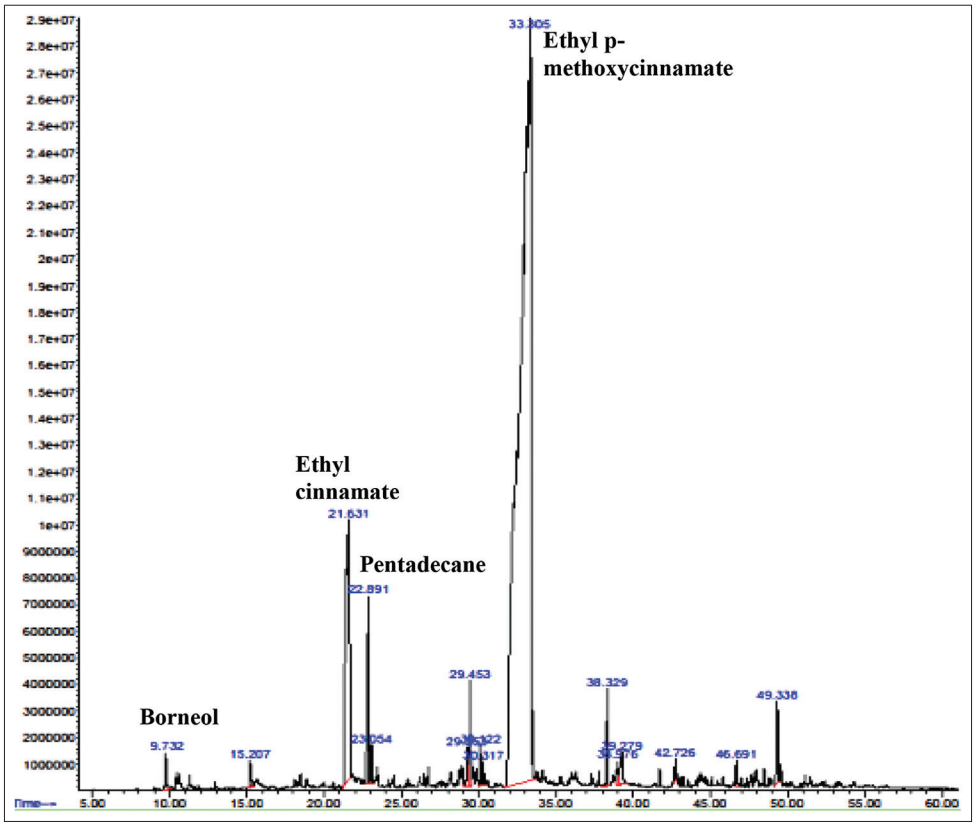

Figure 2: GC MS Chromatogram of *K. galanga* rhizome extract

Table 2: Chemical constituents of leaf extract of *K. galanga.*

SL. No.	Compound name	Area % Mean±SD	Retention time	Formula
1.	Octamethyl cyclotetrasiloxane	11.79±0.2	15.703	$C_8H_{24}O_4Si_4$
2.	Diethyl Phthalate	6.85±0.2	23.987	$C_{12}H_{14}O_4$
3.	Hexadecanal	2.47±0.24	32.710	$C_{16}H_{32}O$
4.	Hexahydrofarnesyl acetone	3.95±0.16	33.628	$C_{18}H_{36}O$
5.	Hexadecanoic acid, methyl ester	2.38±0.28	36.364	$C_{17}H_{34}O_2$
6.	Phytol	1.73±0.12	41.937	$C_{20}H_{40}O$
7	2-(3,4-Dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-chromen-4-one	18.26±0.35	49.955	$C_{18}H_{16}O_6$
8.	2-(3-Hydroxy-4-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one	14.01±0.3	50.682	$C_{18}H_{16}O_6$

Table 3: Chemical constituents of rhizome extract of *K. galanga*.

SL. No.	Compound name	Area % Mean±SD	Retention time	Formula
1.	Borneol	0.40±0.12	9.732	$C_{10}H_{18}O$
2.	Ethyl cinnamate	9.61±0.45	21.631	$C_{11}H_{12}O_2$
3.	Pentadecane	3.13±0.2	22.891	$C_{15}H_{32}$
4.	γ-Muurolene	0.43±0.28	23.056	$C_{15}H_{24}$
5.	8-Heptadecene	0.56±0.25	29.251	$C_{17}H_{34}$
6.	4-Tetradecyne	1.21±0.23	29.454	$C_{14}H_{26}$
7.	Heptadecene	0.35±0.11	30.122	$C_{17}H_{36}$
8.	2-Pentadecanol	0.22±0.12	30.317	$C_{15}H_{32}O$
9.	Ethyl p-methoxycinnamate	80.39±0.85	33.307	$C_{12}H_{14}O_3$
10.	Hexadecanoic acid	0.67±0.12	39.277	$C_{16}H_{32}O_2$

2005; Mohanty *et al.,* 2011; Sahoo *et al.,* 2014). However, there is no report available on GC-MS analysis of leaves extract of *K. galanga*. This is the first report on the chemical constituent analysis of *K. galanga* leaf extract.

CONCLUSION

The present work has been performed to establish the metabolic profiling of *K. galanga* through GC MS analysis, which could have a commercial interest in various pharmaceutical companies for the manufacturing of innovative drugs. The chemical composition of *K. galanga* leaf extract has been done for the first time in this study. EPMC was found to be the major bioactive constituent of *K. galanga* rhizome extract with some other vital components like ethyl cinnamate, pentadecane etc. This primary information will enable further studies on the isolation of bioactive constituents and screening of pharmacological activities.

AUTHOR'S CONTRIBUTION

Suprava Sahoo designed and did the work and wrote the manuscript. Debasis Sahoo helped in reviewing and editing the manuscript. Snehalata Khuntia helped in reviewing and editing the manuscript. Basudeba Kar gave the concept, designed the work, reviewed and edited the manuscript. All authors read and accept the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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