Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (2) : 2023 : 321-326

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i02.022

ETHNOMEDICINAL USES, PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *KAEMPFERIA GALANGA* L. RHIZOME

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(Received 29 November, 2022; Accepted 20 January, 2023)

***Key words**: Ethnomedicinal uses, Antibacterial activities, GCMS analysis, Kaempferia galanga*

Abstract–Zingiberaceae is the largest family of the order Zingiberales containing more than 1600 species and 200 genera among which *Kaempferia* is the medium sized ornamental genus containing near about 60 species mostly distributed throughout the tropics and sub tropics of the world. Being aromatic and rich in diverse bioactive compounds plants of this family are economically, ethnomedicinally and pharmaceutically very important. Apart from their traditional use as spices, food, ethnomedicine, dyes, perfume etc., they have got a huge future potential in new drug development due to their antimicrobial, anti-inflammatory, anti-diabetic, anti-cancer and antioxidant characteristics. In the present study, the ethnomedicinal uses of *Kaempferia galanga* have been documented. In addition to this the antimicrobial properties of this plant have been examined by disc diffusion method. It has been observed that *K. galanga* exhibited remarkable anti-bacterial property against the Gram +ve bacterium *Staphylococcus aureus* than Gram the -ve bacterium *Escherichia coli*. Fifty five compounds have been identified by GCMS analysis. Ethyl p-methoxycinnamate (55.60%) comprised of the maximum area which is followed by other compounds such as Pentadecane (22.46%), 2-Propenoic acid, 3-phenyl-, Ethyl ester, (E)-(7.78%), Eucalyptol (1.11%), Alpha. -Pinene (.11%), Endo-Borneol (0.57%), Germacrene D (0.14%), Camphene (0.07%) and 3-Carene (1.46%) are the major representative compounds.

INTRODUCTION

Zingiberaceae is the largest family of the order Zingiberales containing more than 1600 species and 200 genera among which *Kaempferia* is the medium sized ornamental genus containing near about 60 species. Zingiberaceae is widely distributed through the tropics and sub tropics of Asia, Africa, Australia and America etc. (Jena *et al*., 2020; Pham *et al.,* 2021). The genus *Kaempferia* comprises of various species, which are commonly used as ornaments, spices and as herbal medicines. This genus is mainly distributed in East Asia to China, India, Bangladesh and Southeast Asia like Thailand, Myanmar, Malaysia, Indonesia, Philippines, Laos, Cambodia and Vietnam(Techaprasan *et al*., 2010). The most widespread *Kaempferia* species are *K. galanga*, *K. parviflora*, and *K. rotunda*, *K. augustiflora* and popularly used as traditional medicines for different ailments including infective diseases, wound infection, cough, pain and digestion disorders (Boonsombat *et al*., 2017; Karmakar *et al*., 2016). *K. galanga* has long been used by the local tribes and folk communities of Southern and North-east India including the tribes Kurichiya, Kuruma, Mullukuruma and Malayali; Garo community of Arunachal Pradesh and Assam and the local tribes of Manipur and Meghalaya to treat a number of human ailments like headache, child ear inflammation, cold, whooping cough, indigestion, gastroenteritis, abdominal pain, antidote for snake venom, blood vomiting, diarrhoea, toothache, mouth sores and tongue blisters in infants, body pain, menstrual pain, dandruff, flatulence, abortifacient, inflammation, baldness, arthritis, rheumatism and intestinal wounds (Pham *et al*.,

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2021; Chawengrum *et al*., 2018). It is an important Indian medicinal herb that has a long history of use in the treatment of several kinds of human ailments including cough and cold, fever, headache, pains disorders, skin diseases, rheumatic diseases, arthritis, joint fractures, vertigo, wounds, gastritis. Moreover, the rhizomes of this plant are highly aromatic and have been used widely as spices, in food flavouring, pickles, and cosmetics and in perfumery products (Shetu *et al.*, 2018; Kumar, 2020). The rhizome extract of *Kaempferia galanga* also confirmed the presence ofcineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, p-methoxycinnamic acid and ethyl cinnamate. Several studies have exposed that a potential constituent, named ethyl-pmethoxycinnamate liable to possess assorted pharmacological responses like anti-neoplastic. antimicrobial, mosquito repellent and nematocidal action (Umar *et al.*, 2012).

MATERIALS AND METHODS

Collection of plant specimen

Healthy rhizomes of *K. galanga* were collected from the natural wild habitats of Nilagiri (21.450, 86.815) block of Balasore district in Odisha. Field surveys have been conducted in this region during the flowering season of the plant, i.e., during the month of July to October to know the natural habitat and distribution pattern of this plant. The local Vaidyas or Kavirajs (traditional medicinal practitioners) and other knowledgeable persons of the tribal villages were interviewed during the field visits about the uses of this plant and the plant species was identified with the help of regional flora books (Haines, 1921; Saxena and Brahman, 1994-98).

Plant extraction

The rhizomes of the plant species were collected from the planted plants in the Department of Environmental science, Fakir Mohan University, Balasore, Odisha. The rhizomes were washed properly and then washed in distilled water and cut into slices and air dried at room temperature for 3 to 4 months. Then the completely dried rhizomes were powdered manually. Then the compounds were extracted from the rhizome powder by the Soxhlet apparatus method. 10-gram sample powder was packed by filter paper, tied and soaked in ethanol at 70 °C for 4-8 hr. The ethanol extract was totally evaporated by rotary evaporator to the compounds (10.23±1.02) %. Each weighed dry sample was then reconstituted in 10 ml of ethanol and was stored in the dark at low temperature (4 °C) for qualitative analysis by GCMS and antimicrobial assay.

Field survey

For documentation of the traditional ethnomedicinal knowledge of local communities of the study region, questionnaire was held to record the plant parts used, modes of drug preparation and their doses with respect to any disease. During the field visits, questionnaire programmes were held among the experienced persons and local healers (*Kaviraj* and *Vaidyas*) among which more than 60% were above 50 years old and 40 % were between 30 to 50 years old.

GCMS analysis

GCMS analysis was carried out using Shimadzu GCMS-QP-2020 system equipped with flame ionization detector using SH-Rxi-5 MS capillary column having dimensions 30m (L)x 0.25 mm (dm)x 0.2 μm df (film thickness). The sample (0.1 μl) was

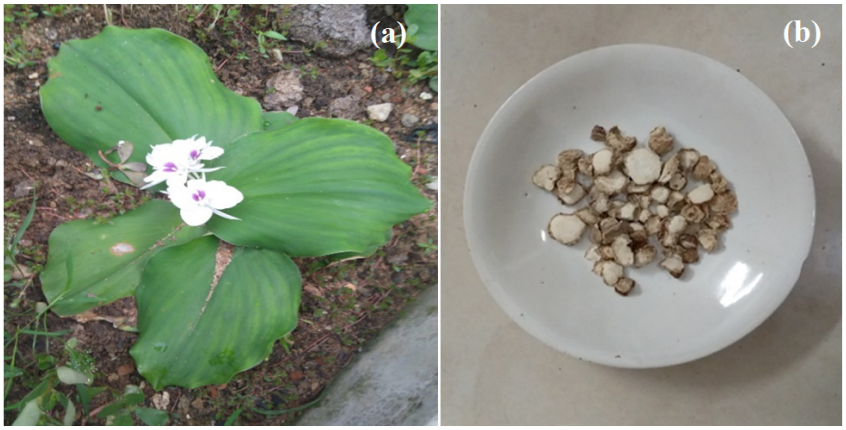

Fig. 1. (a) *Kaempferia galanga* (b) Dried flakes of Rhizome.

injected to the column with the split ratio 1:5. The temperature of the injector and detector was maintained in GC was 260 °C. The oven temperature was initially set at 50 °C with the hold time 1 min then increased to 230 °C at the rate of 5 °C/min with the hold time 5 min then increased to 260 °C at the rate of 15 °C/min with the hold time 10 min. The flow rate of the carrier gas (Helium) adjusted to 1 ml/min.

GCMS analysis was performed using GCMS-QP 2020, Shimadzu system equipped with mass selective detector having ion source temp 200 °C, interface temp 270 °C, solvent cut time 3min threshold 70 ev with the mass range from 50-600 m/z in 54 min.

The compounds were identified by comparison of retention indices (RIs) with those reported in the literature and by comparison of mass spectra with data in NIST library.

Antimicrobial assay

Antibacterial activity of essential oil was assayed by the agar disc diffusion method against the Gram +ve bacterium *Staphylococcus aureus* and Gram –vebacterium *Escherichia coli*. These bacterial strains were procured from the Microbial Type Culture Collection and Gene Bank (MTCC) of CSIR-Institute of Microbial Technology, Chandigarh.

The selected bacteria were cultured in 100 ml nutrient broth culture. In the disc diffusion method nutrient agar was used as culture media and the sterilised discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37 °C for 24 hours. After incubation for 24 hr the zone of inhibition around the disc were measured by centimetre scale. The antibacterial activities were determined by measuring the diameter of zone in cm. This experiment was replicated 3 times to confirm the reproducible result. Sterile blank paper discs were impregnated with essential oil of *K. galanga*, only sterile solvent methanol as solvent control, the sterile distilled water was taken as -ve control and amoxicillin was used as +ve control for comparison of antibacterial activity.

RESULTS AND DISCUSSION

Ethnomedicinal uses

The ethnomedicinal uses of this plant have been documented. *K. galanga* is commonly known as kencur or resurrection lily or aromatic ginger and locally known as Saprakachu. Flakes of dried rhizome are chewed as a post meal digestive and for the treatment of sore throat, bleeding gum and toothache. Dried rhizome flakes are also consumed with betel leaf as flavouring agent. The ash of leaves is applied on swollen breast after child birth for couple of weeks. The rhizome powder is used to avoid dandruff and scab form head traditionally in Arunachal Pradesh and Assam (Basak *et al*., 2010). Rhizome juice is taken orally during malaria, indigestion and fever by the tribes of Koraput (Padhan and Panda, 2015).

Chemical constituents identified by GCMS

Essential oil of *K. galanga* was subjected to GCMS analysis to evaluate its detailed chemical composition. The analysis of essential oil showed the presence of 55 compounds (Table 1) which has been identified by NIST library and literature. Among which the essential oil showed the presence of 21 major compounds accounting for 94.46% of the total peak area. Ethyl p-methoxycinnamate (55.60%) comprised the maximum area followed by Pentadecane (22.46%), 2-Propenoic acid, 3-phenyl-ethyl ester, (E)-(7.78%), Eucalyptol (1.11%), alpha. -Pinene (0.11%), endo-Borneol (0.57%), Germacrene D (0.14%), Camphene (0.07%) and 3-Carene (1.46%) are the major representative compound. Ethyl p-methoxycinnamate has been reported to have many biological activities such as anti-cancer, anti-monoamine oxidase activities, anti-inflammation, hypo pigmentary effect, anti-angiogenesis and anti-tuberculosis effect (Zheng *et al*., 1993: Lakshmanan *et al.*, 2011; Gunasekaran *et al*., 2019; Ko *et al*., 2014). Other reports indicated the main component present in *K. galanga* to be ethyl cinnamate, ethyl p-methoxycinnamate, trans-ethyl cinnamate, methyl cinnamate and ethyl-trans-p-methoxycinnamate (Adianingsih *et al*., 2021; AlSalhi *et al.* 2020).

Antibacterial activity

The essential oil of *K. galanga* exhibited remarkable anti-bacterial activity against the Gram +ve bacterium *Staphylococcus aureus* and Gram -ve bacterium *Escherichia coli*. The EO of *K. galanga* exhibited more antibacterial activity on *Staphylococcus aureus* than the Gram -ve bacterium *Escherichia coli* (Table 2). The extract of *K. galanga* showed antibacterial activity against *Lactobacillus acidophilus* (Saraswati and Septalita, 2017). The extract of *K.galanga* showed moderate antibacterial activity against *Staphylococcus aureus*, *Bacillus aureus, Escherichia coli*, *Pseudomonas aeuginosa* and *Shigella*

Table 1. Chemical constituents of ethanol extract of *Kaempferia galanga* rhizome

S. No.	Compound Name	Retention Time	Area %
1	Ethane, 1,1-diethoxy-	3.150	0.61
2	p-Dioxane-2,3-diol	3.301	0.24
3	Propanenitrile, 2-hydroxy-	3.965	0.06
4	Ethanol, 2-(trimethylsilyl)-	4.362	0.08
5	. alpha. -Pinene	7.177	0.11
6	Camphene	7.569	0.07
7	1,3,5-Cycloheptatriene, 3,7,7-trimethyl-	8.142	0.03
8	Tetraethyl silicate	8.600	0.23
9	. beta. -Myrcene	8.665	0.03
10	3-Carene	9.320	1.46
11	o-Cymene	9.619	0.19
12	D-Limonene	9.745	0.18
13	Eucalyptol	9.837	1.11
14	Undecane	11.757	0.19
15	p-Mentha-1,5-dien-8-ol	13.568	0.09
16	endo-Borneol	13.760	0.57
17	2-Caren-4-ol	14.039	0.12
18	Benzenemethanol,. alpha.,. alpha.,4-trimethyl-	14.169	0.11
19	(+)-cis-Verbenol, acetate	14.878	0.09
20	Benzaldehyde, 3-methoxy-	16.220	0.06
21	4,7,7-Trimethylbicyclo [4.1.0] hept-3-en-2-one	17.861	0.38
22	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethy	17.959	0.08
23	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-meth	20.040	0.06
24	Tetradecane	20.108	0.48
25	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahy	20.317	0.58
26	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-oc	20.554	0.17
27	. gamma. -Elemene	21.108	0.07
28	2-Propenoic acid, 3-phenyl-, ethyl ester, (E)-	21.843	7.78
29	1-Pentadecene	22.172	0.18
30	Germacrene D	22.371	0.14
31	Pentadecane	22.649	22.46
32	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-m	23.165	0.41
33	(-)-. alpha. -Panasinsen	23.285	0.06
34	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimeth	23.369	0.10
35	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dime	23.556	0.25
36	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methyle	24.248	0.08
37	7-Hexadecenal, (Z)-	24.858	0.07
38	Hexadecane	25.017	0.04
39	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabic	25.492	0.07
40	Ethyl p-methoxycinnamate	26.445	56.37
41	11,14-Eicosadienoic acid	26.654	0.26
42	3-Heptadecene, (Z)-	26.810	0.78
43	Linoleyl methyl ketone	26.910	0.44
44	1,8,11-Heptadecatriene, (Z, Z)-	27.020	0.05
45	5-Undecanol, 2-methyl-	27.100	0.11
46	Heptadecane	27.298	1.01
47	Spiro [4.5] dec-9-en-1-ol, 1,6,6,10-tetramethyl-,	28.825	0.03
48	2-t-Butyl-6-[2-hydroxy-2-(4-methoxyphenyl) e	30.825	0.04
49	Phthalic acid, isobutyl tridec-2-yn-1-yl ester	30.938	0.06
50	n-Hexadecanoic acid	32.699	0.11
51	Ethyl 3-(3,4-dimethoxyphenyl) acrylate	32.785	0.14
52	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9	33.082	0.63
53	Aristolene epoxide	35.901	0.16
54	(E)-Labda-8(17),12-diene-15,16-dial	37.929	0.16
55	(E)-Labda-8(17),12-diene-15,16-dial	40.538	0.41

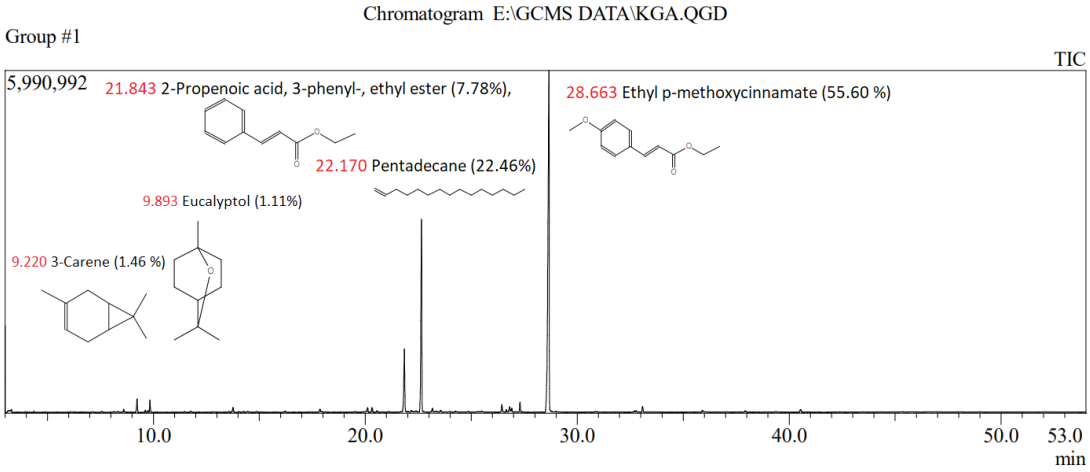

Fig. 2. GC-MS Total Ion Chromatogram of ethanol extract of *Kaempferia galanga* rhizome

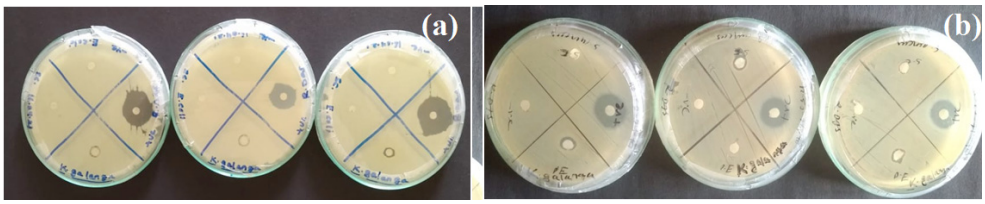

Fig. 3(a). Antibacterial activity against *S. aureus* (b) Antibacterial activity against *E. coli*

dysenterie (Dash *et al*., 2014).

CONCLUSION

In the present study the ethnomedicinal uses of *K.galanga* have been documented. It has been documented the flakes of dried rhizome are chewed as a post meal digestive and for the treatment of sore throat, bleeding gum and toothache. Dried rhizome flakes are also consumed with *paan* as flavouring agent. The essential oil of *K.galanga* showed the presence of 55 compounds which has been analysed by GCMS. Among these compound Ethyl p-methoxycinnamate is the major compound, which possess many biological properties like antibacterial, anti-cancer, anti-monoamine and anti-inflammation activities.

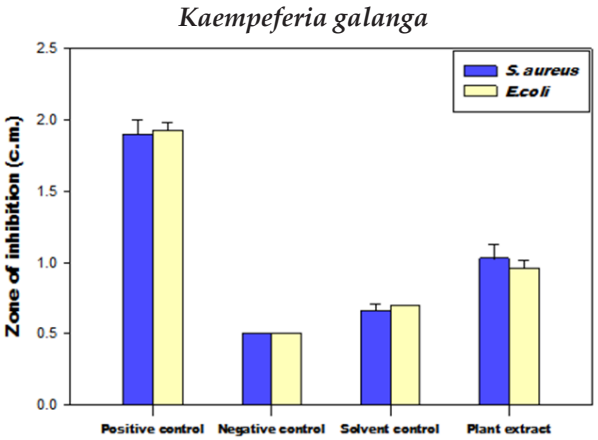

Fig. 4. Size of the zone of inhibition by the ethanolic extract of *K.galanga* rhizome against *S. aureus and E. coli*

ACKNOWLEDGEMENTS

The authors acknowledge Centre of Excellence for Bioresource Management and Energy Conservation Material Development, Fakir Mohan University, Balasore, Odisha(established under OHEPEE grant) for providing necessary research support.

Conflict of Interest

Authors have declared that there is no conflict of interest to publish this manuscript.

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