Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Phytochemical characterization of the ethanolic extract of *Kaempferia galanga* rhizome for anti-oxidant activities by HPTLC and GCMS

Flavius Phrangsngi Nonglang[1], Abhijeet Khale[2] and Surya Bhan[1*]

Abstract

Background: The rhizome of *Kaempferia galanga* (*K. galanga*) was collected from Meghalaya, India, and its ethanolic extract was obtained by freeze-drying or lyophilization process, which was then assessed for its in vitro anti-oxidant activity and phytochemical characterization using high-performance thin-layer chromatography (HPTLC) and gas chromatography-mass spectroscopy (GCMS).

Results: In vitro anti-oxidant activity analysis shows an inhibitory concentration (IC_{50}) value of 1.824 mg/mL and 0.307 mg/mL for, α, α-diphenyl-ρ-picrylhydrazyl (DPPH) and 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays, respectively. Total polyphenol content (TPC) of 23.55 ± 0.5 mg gallic acid equivalent (GAE)/g dry weight of extract and total flavonoid content (TFC) of 100 ± 1.414 mg rutin equivalents (RE)/g dry weight of extract were found. High-performance thin-layer chromatography (HPTLC) analysis shows the best separation of bands at different retention factor (*Rf*) values, when employing the solvent system 2-butanol/1-propanol/water in the ratio of 3:1:1 (v/v/v). Gas chromatography-mass spectroscopy (GCMS) analysis confirms the presence and identification of various phytocompounds, with ethyl p-methoxycinnamate identified as the major active compound.

Conclusion: Freeze-dried ethanolic extract of *K. galanga* (rhizome) possesses anti-oxidant activity. Ethyl p-methoxycinnamate is present as the major bioactive component (about 94.87% of the total area composition), and since it has very important and diverse medicinal properties, a freeze-drying process (lyophilization) can be utilized for its isolation and extraction.

Keywords: *Kaempferia galanga*, Ethanolic extract, Freeze-drying, Anti-oxidants, High-performance thin-layer chromatography, Gas chromatography-mass spectroscopy

Background

A wide variety of medicinal plants found in the tribal regions of the north-eastern part of India have been used extensively for generations for medicinal purposes. One such important medicinal plant is *K. galanga,* an aromatic ginger belonging to the Zingiberaceae family. The plant is widely distributed and prevalent in the tropical and sub-tropical Asian regions of southern China, Malaysia, Thailand, Taiwan, and India [1]. It is commonly known by various local names such as Cekor (Malay), Ekangi (Bengali), Chandramula (Hindi), Kencur (Indonesian), sand/aromatic ginger (English) or Inchmoh (local Khasi name in Meghalaya). *K. galanga* is a perennial, stemless, monocotyledonous aromatic herb formed by thin, moderately branching roots growing from a tuberous stem [2]. The rhizome is fleshy, odorous, and has a dull reddish-brown external skin. The delicate interior is almost whitish to

*Correspondence: bhansurya900@gmail.com
[1] Department of Biochemistry, North Eastern Hill University, Shillong, Meghalaya 793 022, India
Full list of author information is available at the end of the article

light yellowish with a tuberous tip, while the leaves are deep green to darkish green, radiating horizontally along with the ground layer [2]. The flowers on either side of the lip are very delicate, fragrant, white with a purple spot in the axillary fascicles, 6–10 in number [2], blooming for only a short duration, usually between August and September. In folk medicine, the rhizome of *K. galanga* has utility in the treatment of various common ailments such as asthma, rheumatism, indigestion, cold and headache, toothache [3], treatment of phlegm, restoration of internal heat, and also in improving blood circulation [4]. Furthermore, nasal obstruction can be relieved by simply applying the oil prepared from the rhizome around the nasal area [5]. In modern medicine, *K. galanga* has been reported to possess different pharmacological properties like anti-inflammatory [6], anti-apoptotic and anti-carcinogenic activities [7], sedative effects [8], and wound healing activities [9]. The compounds ethyl-cinnamate and ethyl–p-methoxycinnamate are the major bioactive constituents that have been reported in dichloromethane [10], hexane [11], and methanol extract [12].

Kaempferia galanga is found thriving in abundance in the tropical forest of Nongpoh, Ri-Bhoi District, in Meghalaya, India, where the climate is hot and humid, with an average temperature of 21.8 °C and a mean rainfall of 2253 mm per year. The soil in this region is low to extremely acidic, brownish to greyish brown, medium to high in organic carbon content, low to medium in available N, P, and medium to high in P in terms of nutrient content [13]. Traditionally in this area, the rhizome of *K. galanga*, along with the stem of *Tinospora cordifolia* and a small amount of *Zingiber officinale* rhizome (common ginger), is ground into a fine powder and mixed together to prepare a herbal remedy, or mixed with some warm water which was then consumed for the treatment of indigestion, piles, food poisoning, bacterial infections, toothaches, fever, and various other ailments. Due to its unique taste and distinct texture, it is also commonly used in the preparation of various local food delicacies. *Kaempferia galanga* is one of those important traditional medicinal plants that are still underused and comparatively less known in the list of medicinal herbs and spices as issued by the International Organization, despite the variety of useful pharmacological properties it possesses [14].

Most extraction methods employed for sample preparation of *K. galanga* utilized a rotary vacuum evaporator under reduced pressure at a temperature of 40–50 °C [15], or by steam distillation [16]. To examine the variation in phytochemical characteristics and anti-oxidant activity of the ethanolic extract of *K. galanga* rhizome, we utilized a different technique of extraction, i.e. a freeze-drying process at a temperature of −80 to −120 °C (lyophilization). Differences in the technique of sample extraction, contrasting physical and chemical parameters of the area where the plant is grown and cultivated, and variations in the solvent system used in the preparation of the extract of the plant sample may show varied and diverse results in its biological activities and phytochemical constitution. As a result, this research will contribute to the understanding and evaluation of the biological activity of the freeze-dried (lyophilized) ethanolic extract of *K. galanga* (rhizome), including its anti-oxidant activity, phytochemical profiling, characterization, and identification of bioactive components for potential pharmaceutical and therapeutic applications.

Methods

Collection and identification

The rhizome of *K. galanga* was collected from the forest of Nongpoh, Ri-Bhoi district of Meghalaya, and was authenticated by Dr. C. Deori, Scientist-in-Charge, Botanical Survey of India (BSI), Eastern Regional Centre, having the authentication no BSI/ERC/Tech/2019-20/655. The collected rhizome of *K. galanga* was separated from undesirable materials and dried in shade and was ground into a powder form. The powder was then stored in an airtight container and kept in a cool, dark, and dry place until the analysis began.

Plant extraction

Preparation of K. galanga extract: 25 g of fine powder of *K. galanga* (rhizome) was weighed and mixed with 125 mL of 80% ethanol and was thoroughly stirred and aggregated in a magnetic stirrer at room temperature for 24–36 h, after which the mixture was filtered through a No 1 Whatman filter paper. The filtrate was placed in ice-cold −80 °C for 24–36 h and then lyophilized until it became completely dry, using a Scanvac cool safe freeze dryer (−80 to −120 °C internal condenser temperature). The dried crude extract obtained was then weighed and stored in an airtight container at −20 °C until further investigation.

The weight of the extract was used to determine the extraction efficiency (Table 1), and the percentage yield was calculated.

Quantitative analysis of flavonoid and polyphenol contents in K. galanga ethanolic extract

Determination of total flavonoid content

The basic principle behind the use of the aluminium chloride colorimetric method is that $AlCl_3$ forms acid-stable complexes with the C4 keto group and either the C3 or C5 hydroxyl group of flavones or flavonols, resulting in a

Table 1 Extractive yield (%) obtained from the ethanol extract of *K. galanga* (rhizome)

Plant name	Part used	Dry powder weight (W′) g/mL	Dry weight of crude extract (W) g/mL	% Yield (W/W′)
Kaempferia galanga (Ethanol extract)	Rhizome	25 g in 125 mL (0.2 g/mL)	1.82 g from 125 mL 0.01456 (g/mL)	7.28%

yellow colour which can then be quantified using a spectrophotometer at an absorbance range of 410–437 nm [17].

One millimetre of 2% aluminium chloride was added to 1 mL of varying concentrations of rutin (0.25–1.0 mg/mL)/ plant extracts (5 mg/mL) and incubated at room temperature for 10 min. As a blank solution, 1 mL of 2% aluminium chloride was added to 1 mL of distilled water. The flavonoid content of the plant extract was spectrophotometrically quantified against a rutin standard curve at 430 nm. The result was then expressed as mg RE /g dry weight of the extract [17].

Determination of total polyphenols

The Folin–Ciocalteu assay works on the principle of reducing the Folin–Ciocalteu reagent (FCR) in the presence of phenolics to produce molybdenum–tungsten blue, which can then be measured spectrophotometrically at 740 nm. The colour intensity rises in direct proportion to the amount of phenolics in the reaction medium. An oxidation/reduction reaction is the basic mechanism. Sodium carbonate was used to adjust the pH, and an incubation time of 120 min at room temperature is required for the reaction to be completed [18].

One millilitre of the plant extracts (0.5 mg/mL)/standard (0.25–1 mg/mL) to 5 mL of diluted Folin–Ciocalteu reagent (10:1) was added, and ~ 5 min later 4 mL of sodium carbonate (7.5%) was then added in the mixture. The test tubes were then thoroughly vortexed mixed and incubated at room temperature in the dark for two hours. The blue complex formed was then spectrophotometrically quantified against a gallic acid standard curve at an absorbance of 740 nm [18]. Results were expressed as mg GAE/g dry weight of the extract.

In vitro free radical scavenging assay

DPPH radical scavenging activity

Two millilitres of DPPH solution (0.004% in methanol) was mixed with 1 mL of varying concentrations of plant extract (– 0.1-1 mg/mL)/standard ascorbic acid (0.02 - 0.1 mg/mL). The solution mixture was incubated for 30 min at room temperature and allowed to stand in a dark environment. Two millilitres of DPPH added to 1 mL of methanol was used as a control. The absorbance was spectrophotometrically estimated at 517 nm against a blank according to the method of Brand-Williams [19] with slight modification.

$$\%\text{Inhibition} = \text{Abs(control)} - \text{Abs(Sample)} / \text{Abs(control)} \times 100$$

ABTS radical scavenging activity

Two millilitres of ABTS radical cation working solution was allowed to react with 1 mL of varying concentrations of standard ascorbic (0.02- 0.1 mg/mL) or plant extract (0.1- 0.5 mg/mL) and incubated at room temperature in the dark for 4 min. The control was prepared by adding 2 mL of ABTS radical cation working solution to 1 mL of distilled water. The absorbance was spectrophotometrically quantified at 734 nm against a blank according to the proposed method of Re [20] with slight modification.

$$\%\text{Inhibition} = \text{Abs(control)} - \text{Abs(Sample)} / \text{Abs(control)} \times 100$$

HPTLC profiling analysis

Sample preparation and application

1 mg/mL of standard reference compound and 10 mg/mL of ethanolic crude extract were prepared in methanol of chromatographic grade and then filtered through Whatman filter paper No. 1. Prepared extract/standard was applied on a TLC aluminium sheets silica gel 60 F 254 (Merck). One microliter of standard reference in the order of ascorbic acid, caffeine, gallic acid, pyrogallol, quercetin, rutin, and 10 µL of plant crude extract were applied to a 200 × 100 mm plate, each with a band length of 8.0 mm using a Linomat 5 sample applicator set at a dosage speed of 150 nL/s.

Solvent system development

For the separation of compounds in the extract, a variety of solvent system combinations were tried, but a satisfactory resolution and a maximum number of spots were obtained in the following solvent system comprising of 2-butanol/1-propanol/water in the ratio of 3:1:1.

Development of the chromatogram

The plates were developed in a twin through glass chamber of 20 × 10 cm and saturated with the above solvent system combination for 20 min at room temperature up to a distance of 80 mm. The plate was then allowed to dry at room temperature for 5 min. The Rf values and colour of the resolved bands were noted.

Scanning and detection of spots

The air-dried plates were visualized using CAMAG® TLC Visualizer in the ultraviolet (254 nm and 366 nm) and white light. Spots were visible without derivatization at 254 nm (Deuterium lamp, absorption mode, filter K320) and 366 nm (Mercury lamp, fluorescence mode, filter K320) wavelengths, but the best results were observed when the TLC plates were derivatized. Anisaldehyde sulphuric acid (derivatizing agent) was sprayed, and the plates were heated at 100 °C using CAMAG® TLC Plate Heater 3.

Spectrum scanning of the developed plate was performed on a CAMAG® TLC Scanner 4, using a deuterium lamp at 254 nm wavelength and a spectrum speed of 100 nm/s. Baseline correction of the lowest slope with noise of 0.05, peak detection-Gauss (legacy) with a sensitivity of 0.1, a threshold of 0.1, and separation of 1 was used. The chromatogram peaks with their Rf value were noted.

GCMS profiling analysis

Chemical profiling analysis to find out the bioactive compound present in the freeze-dried ethanolic extract of *K. galanga* (rhizome) was performed using GCMS analysis. The study was performed on the Clarus 680 Gas chromatography/Clarus 600 Mass Spectrometer, PerkinElmer Turbo Mass Spectrophotometer (USA) model (GC having a Liquid Autosampler). PerkinElmer Elite Elite-35MS capillary column with a length of 60 m and an internal diameter of 0.2 mm was used. An electron impact technique of 5.0 mV was used. A carrier gas of pure helium gas (99.99%) with a flow rate of 1 mL/min was used. At 280 °C and 0 °C, respectively, the temperatures of the injector and detector were preserved. The oven temperature was initially set at 60 °C with an initial holding time of 1.0 min, at an equilibration time of 2.0 min, and then gradually increased to 350 °C (maximum) with ramp rate 1 of 7.0 °C/min to 200 °C and a holding time of 3.0 min, and ramp rate 2 of 10.0 °C/min to 300 °C and a holding time of 5 min. 1.5 µL of the plant extract (diluted in 1% methanol) was injected (normal velocity) and a split injection technique was used (10:1:1 split ratio). The analysis was conducted and permitted to run for 40 min with a sampling rate of 1.5625 pts/s. Data were assessed for compound identification and quantification using a total ion chromatogram (TIC). The identification of the compound was based on the comparison of their retention indexes (RI) and retention time and also verified by comparison with the library spectra of the National Institute of Standard and Technology (NIST) and published literature data.

Table 2 Quantitative analysis of total flavonoid and polyphenol content

Components	Plants *K. galanga (Eth)* (mg/g)
Polyphenol content (mg GAE/g dry weight extract)	23.55 ± 0.5
Flavonoid content (mg Rutin equivalent/g dry weight extract)	100 ± 1.414

Table 3 IC_{50} value of DPPH and ABTS of the ethanolic extracts of *K. galanga*

Plant name/standards	IC_{50} (mg/mL)	
	DPPH	ABTS
Kaempferia galanga (Eth)	1.824	0.307
Standard (Ascorbic acid)	0.013	0.011

Results

Extractive yield (percentage)

The percentage of extractive yield obtained from the freeze-dried ethanol extract of *K. galanga* (rhizome) was found to be 7.28% (Table 1).

Total flavonoid and polyphenol content

The result obtained shows that the ethanolic extract of *K. galanga* contains total polyphenol content (TPC) of 23.55 ± 0.5 mg GAE/g dry weight of the extract, while the total flavonoid content (TFC) is 100 ± 1.414 mg RE/g dry weight of the extract as detailed in Table 2.

In vitro anti-oxidant activity of the ethanol extract of *K. galanga*

In vitro anti-oxidant activity of the ethanol extract of *K. galanga* shows that the inhibitory concentration (IC 50) value as summarized in Table 3 was 1.824 mg/mL for the DPPH assay (i.e. a maximum % inhibition of 28.05% at 1 mg/mL extract concentration) and 0.307 mg/mL for the ABTS assay (i.e. a maximum % inhibition of 50.2% at 0.5 mg/mL extract concentration), respectively, while the

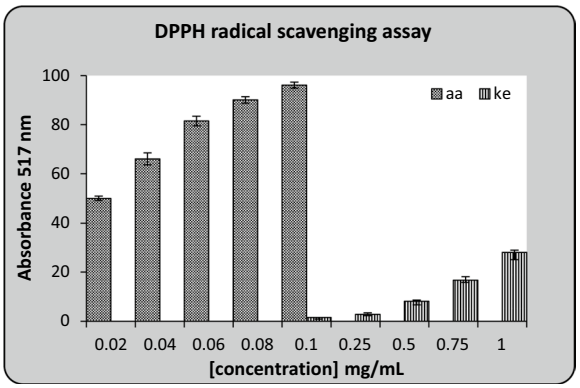


Fig. 1 DPPH free radical scavenging activities

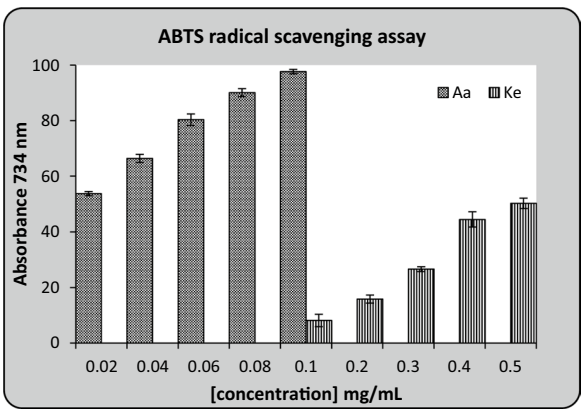


Fig. 2 ABTS free radical scavenging activities

IC 50 value of standard ascorbic acid is 0.013 mg/mL and 0.011 mg/mL for the DPPH and ABTS assays, respectively (Figs. 1 and 2).

HPTLC analysis of the ethanolic extract of *K. galanga*

Fingerprinting profiling and separation of the phytochemical components of the ethanolic extract of *K. galanga* (rhizome) were performed using HPTLC analysis along with standard reference compounds, namely ascorbic acid, caffeine, gallic acid, pyrogallol, quercetin, and rutin.

In various solvent systems, different phytochemicals have distinct Rf values. This difference in Rf values of phytochemicals provides an important clue in determining their polarity, as well as aiding in the selection of a suitable solvent solution for the separation and isolation of pure analyte [21]. To measure the movement of the materials along the plate, the Rf value is noted, which is a measure of the distance travelled by the solute divided by the distance travelled by the solvent. Several combinations of solvent mixtures were used and tried (error and trial method). However, the best separation of the bands was achieved when using the solvent combination of 2-butanol/1-propanol/water in the ratio of 3:1:1 and derivatizing it with anisaldehyde sulphuric acid. The bands of the standard compound as applied on the TLC plates in the order of ascorbic acid, caffeine, gallic acid, pyrogallol, quercetin, rutin, and *K. galanga* ethanolic crude extract (Fig. 3) can be visualized under UV (254 nm and 366 nm) and white light.

The plate scanned at 254 nm for *K. galanga* (Fig. 4) shows 4 peaks, with peak 1 having an Rf value at 0.359 (2.63% area composition), peak 2 having an Rf value of 0.510 (11.58% area composition), peak 3 having an Rf value at 0.556 (8.13% area composition), and peak 4 having an Rf value at 0.776 (having maximum 77.65% area composition) as detailed in Table 4. The plate scanned at 254 nm for standard reference compounds shows ascorbic acid having an Rf value of 0.469, caffeine having an Rf value of 0.544, and pyrogallol having an Rf value of 0.765, whereas the other reference compounds, namely rutin, gallic acid, and quercetin, did not separate in the above solvent system.

Chemical profiling analysis of the ethanolic extract of *K. galanga* by GCMS analysis

Identification of the compound using GCMS analysis was based on the comparison of their retention indexes (RI) and retention time verification by comparison with the library spectra of the NIST and previously published literature data. The GCMS chromatogram (Fig. 5) shows various peaks. The major active compound was identified to be ethyl p-methoxycinnamate, which had a peak at a retention time of 28.79 (mins) and accounted for up to 94.87% of the total area composition of the plant extract. Various other minor compounds were identified, namely dodecane, 1-fluoro (0.918% area composition and peak retention time at 8.038 min), 3-methyl-2-(2-oxopropyl) furan (3.339% area composition and peak retention time at 8.819 min), trieicosane, 1-bromo-11-docosenyliden- (0.033% area composition and peak retention time at 9.879 min), dotriacontyl isopropyl ether (0.049% area composition and peak retention time at 10.409 min), rhodopin (0.023% area composition and peak retention time at 33.673 min) and (1R,4AR,4BS,7S,10AR)-1,4A,7-trimethyl-7-vinyl-1,2,3,4,4A,4B,5,6,7,8,10,10A-DOD (0.068% area composition and peak retention time at 36.30 min) as detailed in Table 5.

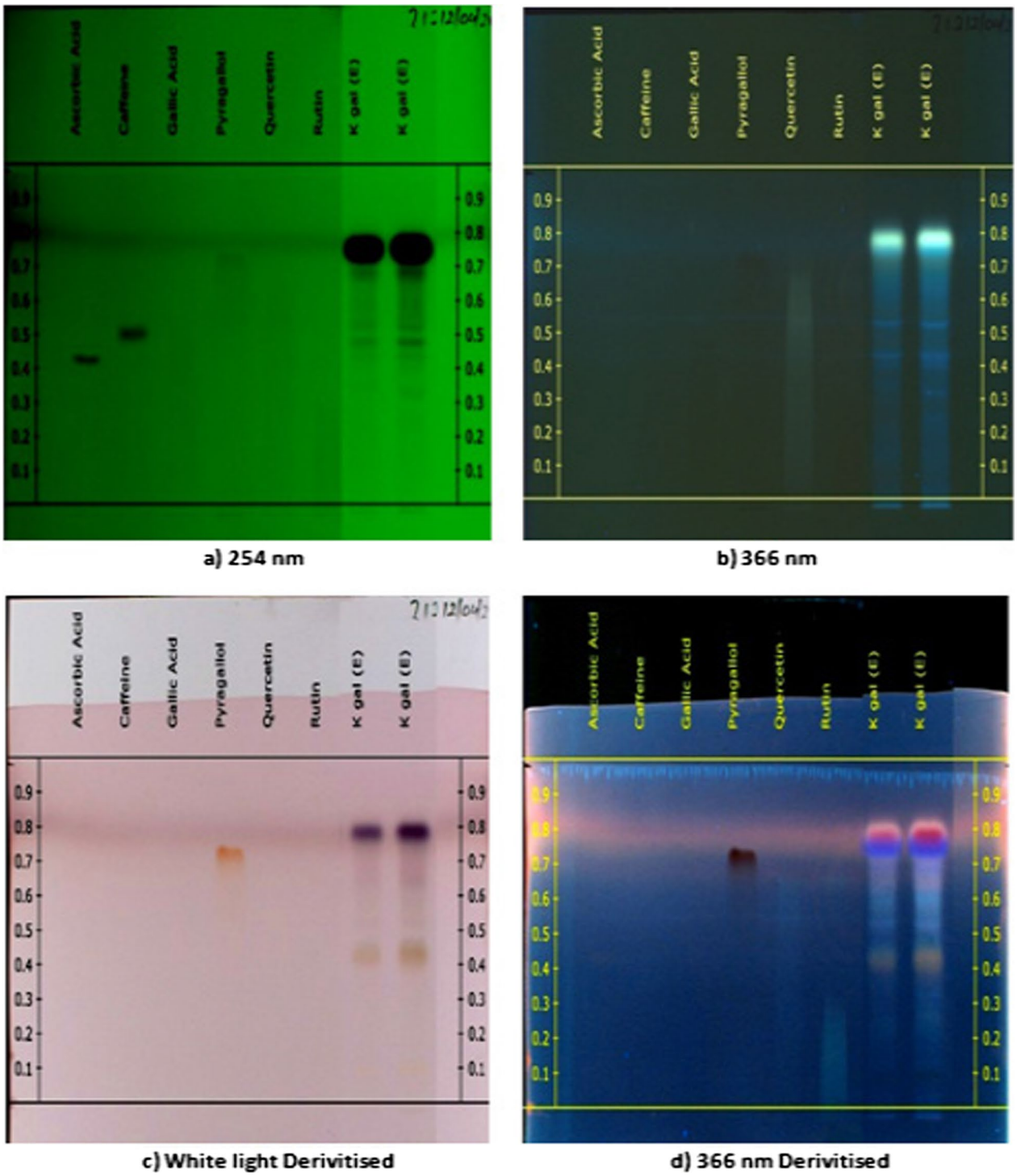


Fig. 3 HPTLC band chromatogram of the ethanolic extract of *K. galanga*

Discussion

The traditional medicinal herbs of India's north-eastern area are abundant and with a growing interest in the identification of natural chemicals for possible pharmaceutical uses; it is critical to objectively assess the medicinal potential of these plants. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that have medicinal and nutritive

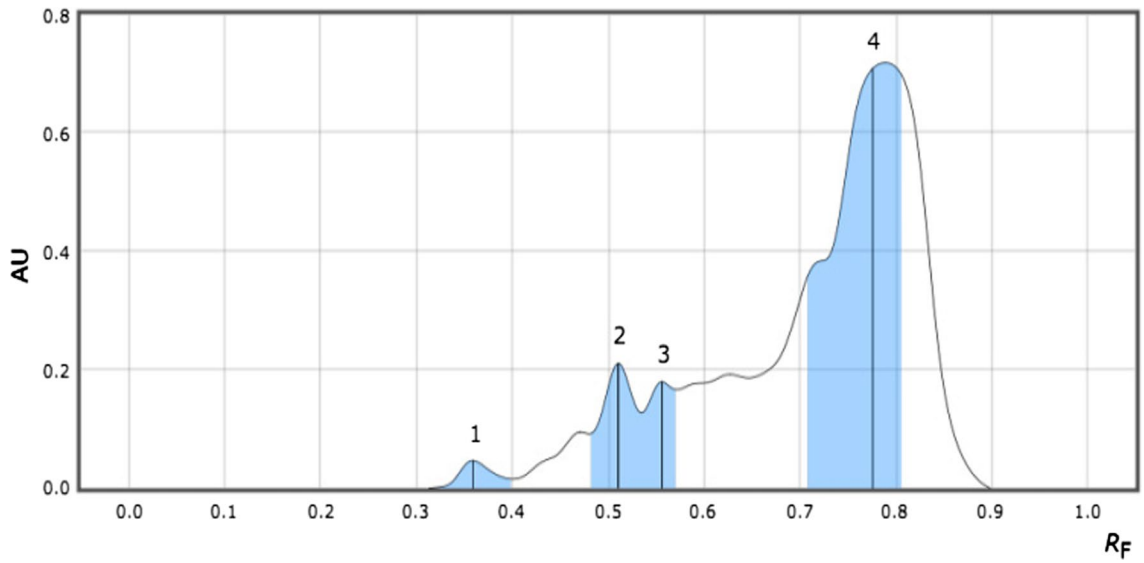


Fig. 4 HPTLC spectrum peak chromatogram of the ethanolic extract of *K. galanga*

Table 4 HPTLC fingerprinting of the ethanolic extract of *K. galanga*

Peak#	Max Rf value	Max height	Area	Area%
1	0.359	0.0466	0.00192	2.63
2	0.510	0.2106	0.00845	11.58
3	0.556	0.1795	0.00594	8.13
4	0.776	0.7085	0.05666	77.65

properties. Phytochemical studies of medicinal plants are critical for both research institutes and pharmaceutical companies in the development of novel drugs [22]. The rhizome of *K. galanga* was selected for these studies due to its common and important usage in local traditional medicines and potent and diverse medicinal properties. Ethanol was used as the extraction solvent since it can dissolve and attract both polar and non-polar compounds. Because of its hydroxyl (OH) group and the high electronegativity of oxygen, it is a highly polar molecule that may form hydrogen bonds with other polar compounds and can also attract non-polar molecules due to the presence of an ethyl group (C_2H_5). It is also less toxic, inexpensive, and readily available. Freeze-drying or lyophilization removes the solvent at a very low temperature, while retaining the compounds that are present in the original state in the plant, concentrates the product, increased their potency compared with their natural form, and limits oxidative changes of metabolites because the oxygen concentration is very low under vacuum [23]. Extraction techniques involving heat such as Soxhlet apparatus, steam distillation, or rotary vacuum evaporator may affect the plant's biological composition and activities since the rate of chemical changes involving thermochemical reactions usually doubles with every 10 °C increased in temperature. As a result, the higher the temperature more probable chemical changes or chemical shifts can be formed [23]. Thus, preparation of plant crude extract using a freeze-drying process or lyophilization (cold extraction) would be considered to most probably be an ideal method. Using the above method of extraction, the percentage extraction yield of the ethanolic extract *K. galanga* (rhizome) obtained was found to be 7.28%. Polyphenols and flavonoids derived from various natural sources have sparked increased interest in research because they can be used as anti-oxidants in the food industry and have a variety of health benefits. Their beneficial effects on human health could be attributed to their free radical scavenging properties, which prevent free radicals from damaging cells [18, 24]. Polyphenols and flavonoids have redox properties that contribute to their anti-oxidant activity [25]. The result obtained shows that the ethanolic extract of *K. galanga* contains TPC of 23.55 ± 0.5 mg GAE/g dry weight of the extract, while the TFC is 100 ± 1.414 mg RE/g dry weight of the extract which indicates the plant-rich source of these phenolic compounds. The in vitro free radical scavenging activities of the ethanolic extract of *K. galanga* were evaluated using the conventional and frequently used DPPH and ABTS model assay. DPPH is a stable synthetic free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule [26]. It is widely used to assess the compound's ability to act as free radical scavengers or radical hydrogen donors and to measure anti-oxidant

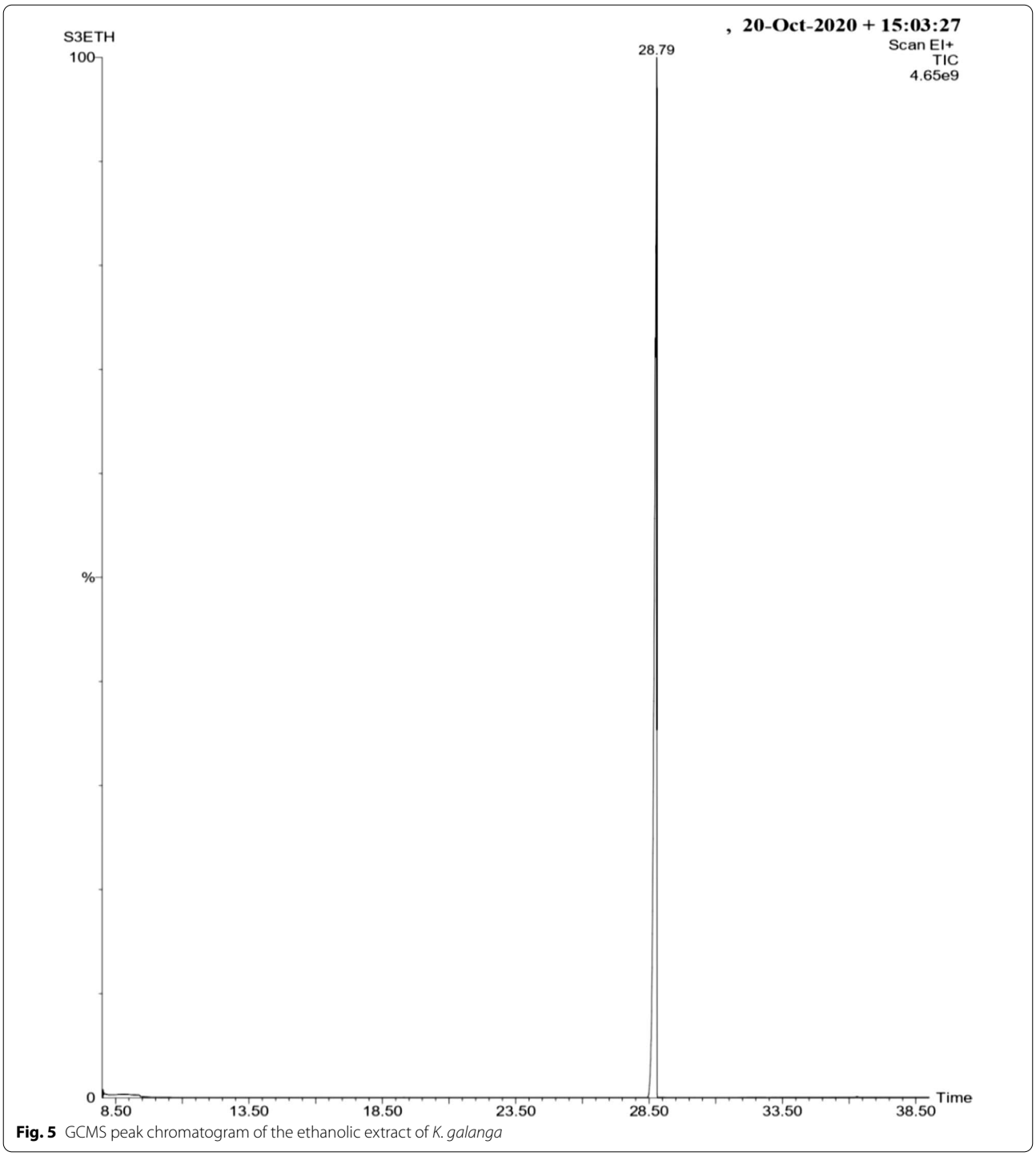
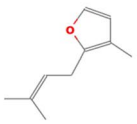
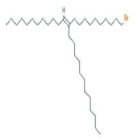
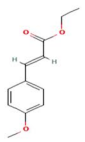
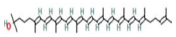
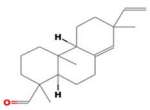


Fig. 5 GCMS peak chromatogram of the ethanolic extract of *K. galanga*

activity. DPPH changes colour from purple to pale yellow, corresponding to the reduced form of DPPH, 2,2-diphenyl-1-picrylhydrazyl (DPPH-H) after receiving an electron from an anti-oxidant molecule, which can be quantified using absorbance changes at 517 nm [26]. On the other hand, in the ABTS assay, pre-formed radical monocation (ABTS*+) is created by oxidation of ABTS with potassium persulfate, resulting in a green–blue ABTS chromophore whose intensity decreases when hydrogen-donating anti-oxidants are present [20]. This change in absorbance intensity can then be quantified at an absorbance of 734 nm. From the above analysis,

Table 5 GCMS library search of the ethanolic extract of *K. galanga*

Sl no.	Retention time (RT)	Height	Area	Area %	NIST	Compound name	Mol. Wt g/mol	Structure
1	8.038	33,787,312	4,425,993.5	0.918	24770	Dodecane, 1-Fluoro	188.32	
2	8.819	15,423,654	16,093,289.0	3.339	24759	3-Methyl-2-(2-oxopropyl) Furan	138.16	
3	9.879	2,152,887	159,092.7	0.033	24432	Trieicosane, 1-Bromo-11-Docosenyliden	569.8	
4	10.409	1,621,721	234,695.1	0.049	24216	Dotriacontyl Isopropyl Ether	508.94	
5	28.79	4,627,524,608	457,253,664.0	94.872	151151	Ethyl p-methoxycinnamate	206.24	
6	33.673	1,661,074	109,381.2	0.023	34358	Rhodopin	554.9	
7	36.30	3,490,355	326,813.4	0.068	174612	(1R,4AR,4BS,7S,10AR)-1,4A,7-Trimethyl-7-Vinyl-1,2,3,4,4A,4B,5,6,7,8,10,10A-DOD	286.5	

the ethanolic extract of *K. galanga* (rhizome) shows improved inhibitory free radical concentration with increasing plant concentration, and when compared with standard ascorbic acid taken as control it shows potent anti-oxidant activity. The important information about the chemical components is typically supplied by the qualitative phytochemical screening of plant extracts for the pharmacological and pathological discovery of new medicines [27]. In recent years, chromatographic and spectral fingerprints have grown in importance in the quality control and standardization of complex herbal medicines [28]. The application and implementation of various chromatographic techniques during the scientific analysis of a plant is a very efficient and effective tool for phytocompound characterization, separation, and identification. A technique for separating compounds in the mixture, HPTLC is a sophisticated instrumental technique that relies on the full capabilities of thin-layer chromatography. The benefits of automation, scanning, full optimization, selective detection principle, minimal

sample preparation, and other features make it a powerful analytical tool for providing chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food products, and other substances, as well as in the analysis of the compound purity [29]. The best separation of the bands was achieved when using the solvent combination of 2-butanol/1-propanol/water in the ratio of 3:1:1 and derivatizing it with anisaldehyde sulphuric acid. The plate scanned at 254 nm for *K. galanga* showed 4 peaks having different Rf values, with peak 4 whose Rf value is at 0.776 having a maximum of 77.65% area composition. GCMS analysis was used to investigate the chemical profile of the ethanolic extract of *K. galanga*, which is a very sensitive, efficient, and powerful chromatographic technique for the identification and quantification of unknown compounds present in a sample. The GC component of the instrument separates the chemical mixtures while the MS component identifies the components at a molecular level. GCMS analysis confirms the presence and identification of several compounds with ethyl-p-methoxycinnamate found as the active constituent and present abundantly in the freeze-dried ethanolic extract of *K. galanga (*rhizome*),* comprising about 94.87% of the total area composition of the plant extract and a peak retention time of 28.79 (mins). Ethyl-p-methoxycinnamate has been previously reported to have anti-tuberculosis activity [30], anti-nematicidal activity [31], mosquito-repellent and anti-larvicidal activity [32], anti-neoplastic [7], anti-microbial potentials [3] and sedative activity [12]. Ethyl-p-methoxycinnamate is also found to be highly cytotoxic to HELA cells, which is a type of cancerous cells [33]. Thus, with ethyl-p-methoxy cinnamate having very important medicinal properties and being found to be present in such a high amount, a freeze-drying process can be utilized for its isolation (Fig. 6).

Conclusion

Traditional herbal medicinal plants are important reserves and treasures of valuable phytocompounds which can be exploited for their potential use in pharmaceutical and therapeutic purposes. Collected from Meghalaya, India, the ethanol extract of *K. galanga* (rhizome) obtained by freeze-drying process shows quite a high percentage yield. In vitro anti-oxidant analysis was accessed, and when compared with standard ascorbic acid as a control, it shows potent anti-oxidant activity. Sufficient polyphenol and flavonoid contents were found to be present. Preliminary HPTLC fingerprinting analysis shows the best separation of bands when employing the solvent combination of 2-butanol/1-propanol/water in the ratio of 3:1:1 and derivatizing it with anisaldehyde sulphuric acid. Four distinct peaks were observed with peak 4, whose Rf (retention factor) value is at 0.77 shown to have maximum 77.65% area composition. GCMS chemical profiling analysis identifies ethyl p-methoxycinnamate as the major bioactive component, accounting for approximately 94.87% of the total area composition of the plant at a peak retention time of 28.79 (mins), as well as the identification of other compounds. Although the anti-oxidant activity may be attributed to the synergistic effects of all the compounds that are present in the ethanolic extract of the *K. galanga* rhizome, however,

Fig. 6 *Kaempferia galanga* rhizome and crude ethanolic extract

since ethyl p-methoxycinnamate is present in such high abundance in the freeze-dried ethanolic extract, it may be proposed that ethyl p-methoxycinnamate may have contributed significantly towards the anti-oxidant potential to an extent. Also, since ethyl p-methoxycinnamate has various diverse medicinal properties, a freeze-drying process (lyophilization) can be utilized for its isolation and extraction.

Abbreviations

K. galanga: *Kaempferia galanga*; Eth: Ethanolic extract; DPPH: α, α-Diphenyl-ρ-picryl hydrazyl; ABTS: 2, 2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; IC 50: 50% Inhibitory concentration; TPC: Total polyphenol content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; RE: Rutin equivalent; HPTLC: High-performance thin-layer chromatography; Rf: Retention factor; GCMS: Gas chromatography mass spectroscopy; RI: Retention indexes; NIST: National Institute of Standard and Technology.

Acknowledgements

The author is grateful to the Department of Biochemistry, NEHU, for providing the infrastructure to conduct the research, Department of Science and Technology (DST) and Department Research Support (DRS) programme for providing research and infrastructural support to the Department, Mr. Prajallendra Kr. Barooah, Guwahati Biotech Park Incubation Centre for providing technical assistance for GCMS analysis. The financial aid provided by Council of Scientific and Industrial Research (CSIR) to Flavius Phrangsngi Nonglang (Letter No - 09/347 (0242)/2019-EMR-1) is also gratefully acknowledged.

Studies involving plants

The rhizome of *K. galanga* was collected from the forest of Nongpoh, Ri-Bhoi district of Meghalaya, India, and was authenticated by Dr. C. Deori, Scientist-in Charge, Botanical Survey of India (BSI), Eastern Regional Centre having the authentication No BSI/ERC/Tech/2019-20/655.

Authors' contributions

FPN was involved in the conception and design of the work, collection of sample, analysis and interpretation of data, and manuscript preparation. AK contributed to the HPTLC design and analysis of sample. SB was involved in the concept of work and review of manuscript. We ensure and hereby declared that all authors have read and approved the manuscript.

Funding

The funding was provided by Council of Scientific and Industrial Research (CSIR) to Mr. Flavius Phrangsngi Nonglang (Letter No-09/347(0242)/2019-EMR-1).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Biochemistry, North Eastern Hill University, Shillong, Meghalaya 793 022, India. [2]Lab Division, Anchrom Enterprises (India) Pvt. Ltd. Mulund (E), Mumbai, Maharashtra 400081, India.

Received: 29 July 2021 Accepted: 22 December 2021
Published online: 08 January 2022

References

1. Techaprasan J, Klinbunga S, Ngamriabsakul C, Jenjittikul T (2010) Genetic variation of Kaempferia (Zingiberaceae) in Thailand based on chloroplast DNA (psbA-trnH and petA-psbJ) sequences. Genet Mol Res 9:1957–1973. https://doi.org/10.4238/vol9-4gmr873
2. Selvam ABD (2012) Pharmacognosy of negative listed plants. Botanical Survey of India, Kolkata
3. Kanjanapothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jitpakdi A, Pitasawat B (2004) Toxicity of crude rhizome extract of *Kaempferia galanga* L. (Proh Hom). J Ethnopharmacol 90:359–365. https://doi.org/10.1016/j.jep.2003.10.020
4. Vittalrao AM, Shanbhag T, Kumari M, Bairy KL, Shenoy S (2011) Evaluation of anti-inflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats. Indian J Physiol Pharmacol 55:13–24
5. Khare CP (2004) Indian herbal remedies: rational western therapy, ayurvedic and other traditional usage, botany. Springer, Heidelberg. https://doi.org/10.1007/978-3-642-18659-2
6. Sulaiman MR, Zakaria ZA, Daud IA, Ng FN, Ng YC, Hidayat MT (2008) Antinociceptive and anti-inflammatory activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. J Nat Med 62:221–227. https://doi.org/10.1007/s11418-007-0210-3
7. Liu B, Liu F, Chen C, Gao H (2010) Supercritical carbon dioxide extraction of ethyl p-methoxycinnamate from *Kaempferia galanga* L. rhizome and its apoptotic induction in human HepG2 cells. Nat Prod Res 24:1927–1932. https://doi.org/10.1080/14786419.2010.490913
8. Ali MS, Dash PR, Nasrin M (2015) Study of sedative activity of different extracts of *Kaempferia galanga* in *Swiss albino* mice. BMC Complement Altern Med 15:158. https://doi.org/10.1186/s12906-015-0670-z
9. Shanbhag TV, Chandrakala S, Sachidananda A, Kurady BL, Smita S, Ganesh S (2006) Wound healing activity of alcoholic extract of *Kaempferia galanga* in Wistar rats. Indian J Physiol Pharmacol 50:384–390
10. Othman R, Ibrahim H, Mohd MA, Mustafa MR, Awang K (2006) Bioassay-guided isolation of a vasorelaxant active compound from *Kaempferia galanga* L. Phytomedicine 13:61–66. https://doi.org/10.1016/j.phymed.2004.07.004
11. Yu JG, Yu DL, Zhang S, Luo XZ, Sun L, Zheng CC, Chen YH (2000) Studies on the chemical constituents of *Kaempferia marginata*. Acta Pharm Sin 35:760–763
12. Huang L, Yagura T, Chen S (2008) Sedative activity of hexane extract of *Keampferia galanga* L. and its active compounds. J Ethnopharmacol 120:123–125. https://doi.org/10.1016/j.jep.2008.07.045
13. Kharlyngdoh A, Zothansiami C, Bora PK, Das PT, Choudhury BU, Singh AK (2015) Characterization and classification of soils in Eastern Himalayan Agro-climatic Region: a case study in Nongpoh micro-watershed of Ri-Bhoi District, Meghalaya. J Indian Soc Soil Sci 63:24–29
14. Nag S, Mandal S (2015) Importance of ekangi (*Kaempferia galanga* l.) As medicinal plants- a review. Int J Innov Res Rev 3:99–106
15. Jagadish PC, Latha KP, Mudgal J, Nampurath GK (2016) Extraction, characterization and evaluation of *Kaempferia galanga* L. (Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats. J Ethnopharmacol 194:434–439. https://doi.org/10.1016/j.jep.2016.10.010
16. Omar MN, Razman S, Nor-Nazuha MN, Nazreen MNM, Zuberdi AM (2013) Supercritical fluid extraction (SFE) of Malaysian wild ginger *Zingiber puberulum* inflorescence. Orient J Chem 29:89–92
17. Lamaison J, Carnat A (1990) Teneur en principaux flavonoiedes des fleurs et des feuilles de Crataegus monogyna Jacq. et de Crataegus laevigata (Poiret) DC. (Rosaceae). Pharm Acta Helv 65:315–320
18. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American J Enol Vitic 16:144–158
19. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol 28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation

decolorization assay. Free Radic Biol Med 26:1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
21. Sharma V, Pallival R (2013) Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of *Moringa oleifera* pods. Int J Green Pharm 7:41–45. https://doi.org/10.4103/0973-8258.111607
22. Wadood A (2013) Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem 02:1–4. https://doi.org/10.4172/2161-1009.1000144
23. Eloff J (2015) How will freeze-drying affect the plant extract? In: Research Gate. https://www.researchgate.net/post/How will freeze-drying affect the plant extract. Accessed 22 Jul 2021
24. Annegowda H, Nee C, Mordi M, Ramanathan S, Mansor S (2010) Evaluation of phenolic content and antioxidant property of hydrolyzed extracts of *Terminalia catappa* L. Asian J Plant Sci 9:479–485. https://doi.org/10.3923/ajps.2010.479.485
25. Butkhup L, Samappito S (2011) *In vitro* free radical scavenging and antimicrobial activity of some selected Thai medicinal plants. Res J Med Plant 5:254–265. https://doi.org/10.3923/rjmp.2011.254.265
26. Kedare SB, Singh RP (2011) Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48:412–422. https://doi.org/10.1007/s13197-011-0251-1
27. Talukdar A, Choudhury MD, Chakraborty M, Dutta B (2010) Phytochemical screening and TLC profiling of plant extracts of Cyathea gigantea (Wall. Ex. Hook.) Haltt. and Cyathea brunoniana. Wall. ex. Hook (Cl. & Bak.). Assam Univ J Sci Technol 5:70–74
28. Gong F, Wang B, Chau F, Liang Y (2005) Data preprocessing for chromatographic fingerprint of herbal medicine with chemometric approaches. Anal Lett 38:2475–2492. https://doi.org/10.1080/00032710500318338
29. Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S (2011) High-performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery. Pharm Methods 2:71–75. https://doi.org/10.4103/2229-4708.84436
30. Lakshmanan D, Werngren J, Jose L, Suja KP, Nair MS, Varma RL, Mundayoor S, Hoffner S, Kumar RA (2011) Ethyl p-methoxycinnamate isolated from a traditional anti-tuberculosis medicinal herb inhibits drug-resistant strains of *Mycobacterium tuberculosis* in vitro. Fitoterapia 82:757–761. https://doi.org/10.1016/j.fitote.2011.03.006
31. Choi IH, Park JY, Shin SC, Park IK (2006) Nematicidal activity of medicinal plant extracts and two cinnamates isolated from *Kaempferia galanga* L. (Proh Hom) against the pinewood nematode, *Bursaphelenchus xylophilus*. Nematology 8:359–365. https://doi.org/10.1163/156854106778493402
32. Ahn Y, Kim N, Byun S, Cho J, Chung K (2008) Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species. Pest Manag Sci 64:857–862. https://doi.org/10.1002/ps.1557
33. Kosuge T, Yokota M, Sugiyama K, Saito M, Iwata Y, Nak-kura M, Yamamoto T (1985) Studies on anticancer principles in *Biota orientalis* and *Kaempferia galanga*. Chem Pharm Bull 33:5565–5567. https://doi.org/10.1248/cpb.33.5565

Publisher's Note