International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.2, pp 422-431, 2014-2015

HPLC analysis and antioxidant activities of hydroethanolic leaf extract of *Kaempferia galanga* Linn.

B. Kaushita, C. Lekhya Priya, K.V. Bhaskara Rao*

Molecular and Microbiology Research Laboratory, School of Bio Sciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

Abstract: The aim of the present investigation was to assess the *in vitro* antioxidant potential, antibacterial activity, haemolytic activity, phytochemical screening and High Performance Liquid Chromatography (HPLC) analysis of hydroethanolic extract of *Kaempferia galanga* leaves. To determine the antioxidant potential of the extract, DPPH radical scavenging, β-carotene linoleic acid, metal chelating and anti-Lipid peroxidation activities were performed. To analyze the effect of extract on human erythrocytes hemolytic assay was carried out. Preliminary phytochemical screening was performed to detect the presence of phytochemical compounds such as alkaloids, flavonoids, terpenoids, saponins, carbohydrates, phenols, phytosterols and tannins. Extract was also tested for the antibacterial activity by agar well diffusion method. The test organisms used were *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Klebsiella pneumonia*. Extract showed antioxidant potential with IC_{50} values of 732µg/mL for β carotene linoleic acid assay, 531µg/mL for anti-Lipid peroxidation assay and 635µg/mL for metal chelating assay respectively. Extract exhibited moderate antimicrobial activity with inhibition zones of 19 mm, 14 mm and 12 mm diameter against *K. pneumonia*, *P. aeroginosa* and *S. aureus.* Whereas, hydroethanolic extract showed no hemolytic activity against human erythrocytes. Further, Fourier Transform Infra Red (FT-IR) analysis resulted in the identification of functional groups. HPLC analysis detected the presence of Genistein, Ferulic acid, Coumaric acid and Butein. Thus, it was concluded that *K. galanga* extract has good antioxidant property and in future the bioactive compound with antioxidant nature in the extract could be isolated and used as a remedy for free radical associated human diseases.
Key words: *Kaempferia galanga*; hydroethanolic extract; anti-Lipid peroxidation assay; β-carotene linoleic acid assay; hemolytic assay; HPLC analysis.

Introduction

Free radicals are the molecules with an unpaired electron in the outermost orbital which makes them unstable and highly reactive towards other molecules, if they come in contact with each other. In living organisms, the free radicals such as superoxide and nitric oxide and the products of their reactions regulate many processes, such as coordination of vascular tone and blood pressure. They play an essential role in the intermediate metabolism of many biological compounds and also are messengers in oxidation reduction signaling in the system[1].

The chemical reactions occurring in our body inevitably produces free radicals[2-3]. Many of these are necessary for the intracellular killing of bacteria by phagocytosis. Thus free radicals are effective in protecting the body against infectious organisms[4]. But when the free radicals level increases beyond the threshold in the body, they can attack DNA, leading to dysfunction, mutation, myocardial infarction, tumors, diabetes, infertility, gastric mucosal injury, brain dysfunction and cancer. They can attack enzymes and proteins, damaging the normal activity of the cell by producing a chain of reactions[5]. Such damage in membranes which

lines blood vessels can often lead to mortis and thickening of the arteries, opening the avenues for heart attacks and stroke[6]. Oxidative stress, the frequent attack by oxy radicals and ROS initiates and promotes many major diseases in the body. Free-radicals attacks on collagen and the muscle fibers causing the cross-linking of protein molecules, resulting in stiffness in the tissue. The free radicals are responsible for ageing because cells accumulate free radical damage over time. Atherosclerosis is also attributed to free-radical induced oxidation of many of the chemicals making up the body[7].

An antioxidant is a substance that prevents the oxidation of a substrate, when present in low concentrations along with the substrate that can be readily oxidized[8]. Antioxidants are generally phenols or polyphenols which fulfill the oxy radicals and the substrate- derived radicals by donating a hydrogen atom or an electron to the free radical and thus conferring the antioxidant activities of the system. Plants contain numerous free radical scavenging molecules which are rich in antioxidants and shows different activities[9].

K. galanga belongs to the family Zingiberaceae commonly known as resurrection lily, it is a short-stemmed herb which has flat, green, round leaves that measure 3-6 inches across. They have white flowers and grow to about 1 inch breadth. It is mainly seen in tropical areas of Africa, and in Southeast Asia[10]. It is cultivated in Southeast Asia especially in Indonesia, southern China, Taiwan, Cambodia and India. All species in this genus are used to flavor rice and as a medicine. It is used to make incense in Japan. In Thailand, the root and leaves are using in curries as a flavoring, and the plant is used as a medicine, as well[11]. *K. galanga* is used in the folk medicines of Asia as an expectorant and carminative. A tea made of the leaves is employed for sore throat, swellings, rheumatism, and eye infections in India[12]. In Thailand, the crushed root mixed with whiskey is applied to the head as a headache treatment[13]. The stimulant and tonic properties of the plant immediately reduce fever and inflammation. The root of *K. galanga* is considered warm, fragrant, and pungent, and very beneficial for the lungs. It is used for cold, vomiting, diarrhoea, intestinal parasites, and toothache[14]. The plant extracts of *K. galanga* also have been found to have anti-inflammatory, analgesic, nematicidal, mosquito repellent, larvicidal, vasorelaxant, sedative, antineoplastic, antimicrobial, antioxidant, antiallergic and wound healing properties[15].

Since there were no studies on the hydroethanolic extract of *K. galanga*, the present investigation was aimed to determine the antioxidant, antimicrobial, hemolytic activities and phytochemical detection of hydroethanolic extract of *K. galanga* leaves.

Materials and Methods

Chemicals and Medias

Ethanol, carbinol, chloroform, β-carotene, linoleic acid, hydrogen peroxide, ferrous sulphate, 2,2-diphenyl-1 picrylhydrazyl radical (DPPH), Folin Ciocalteu's phenol reagent and Tween 40,ferric chloride, sodium carbonate, ascorbic acid, ferrozine, thiobarbituric acid and trichloroacetic acid were obtained from Sisco Research Laboratories Pvt.Ltd. Nutrient agar, nutrient broth, Muller Hinton agar, potato dextrose agar and Sabaraud's dextrose agar were obtained from HiMedia Laboratories Pvt.Ltd.

Plant material collection and authentication

K. galanga leaves were collected from a local garden in Kerala, India during the month of August, 2013. The taxonomic identification and authentication of the plant was done by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre (PARC), Chennai. The herbarium of the plant with the accession number PARC/2013/1445 was further maintained in the laboratory for future reference.

Processing of the Plant material

Fresh leaves of *K. galanga* were shade dried and then made into fine powder using mechanical grinder. Hydroethanolic extract of the leaves was prepared by taking 40 grams of the powdered plant material into the Soxhlet apparatus with 300 mL of ethanol (70%v/v) and the extraction was carried out for three days. The resulted extract was evaporated to dryness using rota evaporator. Thus obtained powder was stored in refrigerator at 4 ºC for further studies.

Phytochemical screening

Phytochemical screening was performed for alkaloids, saponins, tannins, flavonoids, carbohydrates and terpenoids using standard procedures[16].

Estimation of the total phenolic compounds

Total phenolic content (TPC) of the extract was estimated following the procedure of Velioglu et al., 1998[7]. A volume of 100µL of each concentration (125, 250, 500 and 1000 µg/mL) prepared was added to a mixture of 2.5 mL of Folin-ciocalteau reagent (1:10 dilution using sterile distilled water) and 2 mL of 7.5% Na_2CO_3. The resulting solution was incubated for 15 mints at 45°C. Two mL of Na_2CO_3 solution (2ml of 7.5% Na_2CO_3 in 2.60 ml of distilled water) was used as a blank. The absorbance readings were taken at765 nm in UV-Vis spectrophotometer. Gallic acid was used as standard. Data was calculated as gallic acid equivalence in µg. Data for each concentration was recorded in triplicates.

Antioxidant activities

DPPH radical scavenging activity

DPPH radical scavenging activity was measured as per the procedure of Shimada et al., 1992 [17]. One mL of 0.2 mM DPPH solution in carbinol was added to 1 mL of plant extract of different concentrations (125, 250,500 and 1000 µg/mL) and then the mixture was shaken vigorously and left in the dark at room temperature for 30 mints. The absorbance of the resultant solution was measured at 517 nm in a UV-Vis spectrophotometer. While the mixture of carbinol and DPPH (2:1 v/v) was used as control and carbinol alone was used as blank. The DPPH radical-scavenging activity was calculated as follows:

$$\% \text{ DPPH radical scavenging} = [(A_c - A_t) / A_c] \times 100$$

Here, A_c is the absorbance of the control; A_t is the absorbance of tested sample

β-carotene bleaching inhibition assay

In this assay, antioxidant activity was determined by measuring the inhibition of conjugated dienehydroperoxides arising from linoleic acid oxidation[18]. A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 2 mg of β-carotene was dissolved in 1 mL chloroform, and 25µL of linoleic acid and 200 µL of Tween-40 were added as emulsifier since β-carotene is not water soluble. Chloroform was completely evaporated using hot air oven. Then, 100 mL of distilled water was added. 3000 µL of this reaction mixture was dispensed into test tubes, and 100 µL of the plant extracts at various concentrations (125, 250, 500 and 1000 µg/mL) were added. The emulsions were incubated for 120 minutes at 50˚C .The same procedure was repeated with a experimental control and a blank. After this incubation time, the absorbance of the mixture was measured at 470 nm.

$$\% \text{ Anti linoleic acid peroxidation} = [(AS_{120} - AC_{120}/AC_0 - AC_{120})] \times 100$$

Here, AS_{120} = Absorbance of test sample after incubation for 120 mints.
AS_{120}= Absorbance of control after incubation for 120 mints.
AC_0-= Absorbance of control before incubation for 120 mints.

Metal chelating activity

The metal chelating activity of the extract for ferrous ions was measured by fallowing the method of Dinis et al., 1994[19]. The reaction mixture containing 0.5 mL of plant extract (125, 250, 500 and 1000 µg/mL concentrations) 1.6 mL of milliQ water, 0.05 mL of $FeCl_2$ (2 mM) and 0.1 mL of Ferrozine (5 mM) was incubated at 40°C for 10 min and the absorbance was measured at 562 nm. The chelating activity of the extract at different concentrations was calculated as:

$$\% \text{ Chelating Activity} = [(A_1 - A_2) / A_0] \times 100$$

Where A_0 = Absorbance of the control (without extract); A_1=Absorbance of reaction mixture and A_2=Absorbance without $FeCl_2$.

Anti-Lipid peroxidation inhibition assay

This assay was used to estimate the degree of lipid peroxidation of the plant extract by fallowing the method of Mandal and Chatterjee, 1980. A 10% homogenate was prepared from freshly excised liver of goat using cold phosphate buffer saline (pH 7.4). A volume of 0.1 mL of the plant extract (125, 250, 500 and 1000 µg/mL concentrations) and 2.8 mL of the liver homogenate were added to 0.1 mL of (50 mM) ferrous sulphate. After 30 min of incubation, 0.1 mL of the reaction mixture was mixed with 1.5 mL of 10% TCA and further incubated for 10 min. It was then filtered and the supernatant was added in a tube having 1.5 mL of 0.67% TBA (in 50% acetic acid) and placed in a boiling water bath for 30 min. The color developed by the different concentration was measured at 535 nm[20]. Anti-lipid peroxidation was assessed by using the following formula:

% Lipid peroxidation inhibition= $[(A_i - A_s) / (A_i - A_c)] \times 100$
Here, A_i = Absorbance of Fe2+ induced peroxidation; A_s = Absorbance of test sample;
A_c = Absorbance of control

Antimicrobial studies

Bacterial isolates

The test bacterial isolates used in this study were *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 2063, *Pseudomonas aeroginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 13883. Antibiotic discs used were Vancomycin (30µg/disc), Cotrimoxazole (23.75µg/disc), Ciprofloxacin (5µg/disc), Chloromphenicol (30µg/disc) and Piperacillin (100µg/disc). The test organisms were inoculated in nutrient broth (pH 7.4.) for 16 hours to obtain fresh culture.

Positive and Negative control

Vancomycin (30µg/disc), Chloromphenicol (30µg/disc), Ciprofloxacin (5µg/disc), Piperacillin (100µg/disc), Cotrimoxazole (23.75µg/disc) were used as positive controls against *S. aureus, K. pneumonia, B. cereus, P. aeroginosa* and *E. coli.* Sterilized distilled water was used as negative control.

Agar well diffusion method

The bacterial isolates were uniformly swabbed onto the Mueller Hinton agar (MHA) petridishes using sterilized cotton swabs. Wells were made in each of these MHA plates using sterilized cork borer of 7 mm diameter. A volume of 100 µl of the hydroethanolic extract of four different concentrations (200, 400, 800 and 1000 µg/ mL) were prepared from the main stock and 100 µl of negative control was poured in the separate wells. The standard antibiotic discs were placed in each of the MHA plate as positive control. Plates were incubated at 37°C for 24 hrs. Each experiment was performed in triplicates. After 24 hrs, the MHA plates were observed for the inhibition zone. The experiment was performed in triplicates[21].

Hemolytic activity

Hemolytic activity was performed by spectrophotometer method described by Yang et al., 2005[22]. A volume of 0.5mL of the red blood cells suspension was mixed with 0.5 mL of the plant extracts (125, 250, 500 and 1000 µg/mL concentrations) in phosphate buffer saline. The mixtures were then incubated for 30 min. After incubation, it was centrifuged at 1500 rpm for 10 min in a laboratory cooling centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal haemolytic controls. Tests were done in triplicates for each concentration. The level of percentage hemolysis by the extracts was calculated according to the following formula:

% hemolysis= $(A_1-A_2 / A_0-A_2) \times 100$

Where, A_1 is the absorbance of test sample; A_2 is absorbance of the control (saline control); A_0 is the absorbance of the control (water control)

FT-IR Analysis

The dried leaves of *K. galanga* were grounded into fine powder and 2 mg of the sample was mixed with 200 mg KBr (FT-IR grade) and pressed to make into a pellet. The sample pellet was placed into the sample

holder and FT-IR spectra were recorded in the range 4000-4500 cm^{-1} in Mattson 1,000 FT-England FTIR system.

HPLC analysis

HPLC analysis for the identification of polyphenols present in the hydroethanolic extract was done using Waters 2487 HPLC system which consists of dual λ detector and a Waters 1525 binary pump accompanied by a Waters Symmetry® C18 column (5 µm, 4.6 × 150 mm) with Waters Sentry™ universal guard column (5 µm, 4.6 × 20 mm). Comparision was made between sample retention times with retention times of the standards. Gradient elution was carried out at 35ºC using two solvents i.e., Solution A (50 mM sodium phosphate in 10% methanol; pH 3.3) and Solution B (70% methanol).The programme is as follows: : 0–15 min – 100% of Solution A; 15–45 min – 70% of Solution A; 45–65 min – 65% of Solution A; 65–70 min – 60% of Solution A; 70–95 min – 50% of Solution A; 95–100 min – 0% of Solution A. Gradient elution was carried out with an injection volume of 20µL (10mg/mL extract). Identification of various phenolic compounds were observed at different wavelengths such as 250 nm-benzoic acids, isoflavones and most anthraquinones; 280 nm-flavones, flavanones, catechins, the aflavins and some anthraquinones; 320 nm-cinnamic acids, most flavones and chalcones; 370 nm-flavonols; 510 nm-anthocyanins[23].

Statistical analysis

The results of the present study were expressed as mean± standard deviation and each experiment was performed in triplicates using Graph pad Prism v.6.00 (La, Jolla, CA, USA).

Results and Discussion

The leaves of *K. galanga* were selected for the present study based on numerous medicinal uses in traditional medicinal system[12]. There are reports on antioxidant and antimicrobial activities of *K. galanga* earlier for the whole plant and its rhizomes but not for the leaves[24-25]. Thus in our study, we tried to identify the antioxidant as well as antimicrobial properties of the leaves.

Yield of the plant extract

The yield of hydroethanolic extract of *K. galanga* was calculated and expressed in percentage of which extract showed the maximum yield of 41%.

Phytochemical screening

Phytochemicals are essential secondary bioactive compounds that confer medicinal value to the plant and are responsible for the controlled function of the body[26].Terpenoids manifest predominant anti-inflamatory, anticancer, anti-malarial and antibacterial and activities[27] and plant terpenoids are extensively used for their aromatic qualities and thus are used in attracting mites. Alkaloids, another crucial bioactive components are employed as anaesthetic agents[28-29]. Flavonoids are seen to inhibit the enzyme topoisomerase and also effective in arresting the mucosa of intestine against the reactive oxygen species. The present study on *K. galanga* leaf extracts showed the presence of phenols, flavonoids, alkaloids, terpenoids, saponins, carbohydrates and absence of proteins, oils, fats and tannins.

Total phenolic content estimation assay

Phenolic compounds are the major contributors of the antioxidant properties in plants and they are most profuse dietary antioxidants[30-31] that prevent the cellular components against oxidative damage[32]. Total phenolic content of the hydroethanolic leaf extract was measured and expressed as Gallic acid equivalents per gram of extract. Phenolic content was maximum for the extract and the increase was dose dependent with 28 mg GAE/g of extract as shown in Figure. 1.

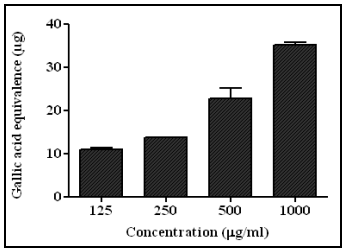

Figure.1 Total phenolic content estimation assay

DPPH Radical Scavenging activity

The free radical scavenging activity of the extract is related with the ability to donate electrons and the conformational changes of the antioxidant compound of the extract. DPPH free radical method is based on the transfer of electrons resulting in a violet colored solution. The free radical is usually stable at room temperature and is broken down to give a colorless ethanol solution in the presence of any antioxidant molecule at 517nm[33].

The DPPH radical scavenging activity of the leaf extract was found to increase with increasing concentrations in a dose dependent manner. The results were expressed as percentage inhibition of DPPH. Free radical scavenging effects results were elucidated as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% in 30 mints (IC_{50}) which resulted in 91% of radical scavenging with an IC_{50} value of 443µg/mL was shown in Figure. 2.

β-carotene-linoleic acid activity

β-carotene is a fat soluble and lipophilic terpenoid. A free radical, which is controlled in the biological systems by some of the essential enzymes that possess antioxidant activity such as superoxide dismutases[34]. In the absence of antioxidants, β-carotene undergoes rapid bleaching and decolorisation from the formation of hydroperoxides by linoleic acid oxidation. The addition of an antioxidant can prevent the bleaching of orange color. The plant extract has been observed to show good antioxidant activity with 732 µg/mL of IC_{50} depicting a considerable increase with increasing concentrations and is shown in Figure. 3.

Anti-Lipid peroxidation assay

Peroxidation appears to be important in many human diseases including atherosclerosis and deleterious effect in primary tissue damage and traumatic brain damage. Oxidative stress can harm proteins and DNA which are more significant targets of injury than lipids, and this lipid peroxidation often is the last stage in the injury process. Application of simple diene-conjugate and thiobarbituric acid (TBA) assays to human tissues and body fluids has shown considerable results of lipid peroxidation[35-37]. Thus lipid peroxidation is an indicator of damaged tissues that is induced by reactive oxygen species which finds its use in various ailments and the most commonly applied assay for lipid peroxidation is Thiobarbituric acid (TBA) test. The sample when heated with TBA at low pH gives a pink colour (a malodialdehyde adduct) which is measured at 532nm. The lipid peroxidation for *K.galanga* extract measured by the thiobarbituric acid assay (TBA) and depicted 80% of Lipid peroxidation inhibition with IC_{50} value of 531µg/mL shown in Figure. 4.

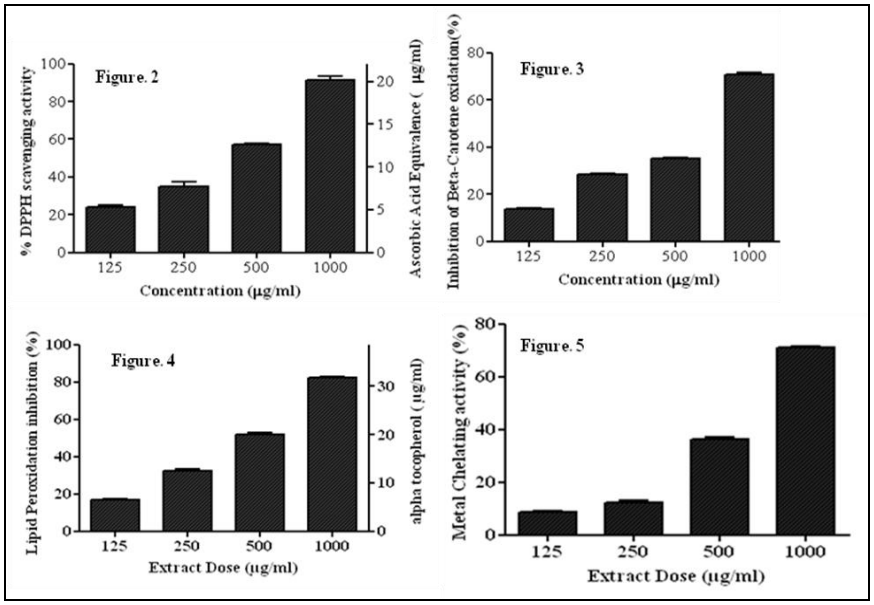

Figure.2 Percentage DPPH free radical scavenging activity of the extract; Figure.3 β-Carotene-linoleic acid activity of the extract; Figure.4 Percentage lipid peroxidation inhibition activity of the extract; Figure.5 Metal Chelating activity of the extract

Metal Chelating assay

Ferrozine that is predominantly used in this assay is an essential component in metal chelating assay can quantitatively form complexes with transition metal ions, in the presence of chelating agents, this complex formation is disturbed by decreasing the red color complex formation. Measurement of color reduction allows the estimation of the chelating activity of existing chelator. Fe^{2+} (transition metal ion) possess the ability to move single electrons and thus allow the formation and propagation of radical reactions[9]. They mainly avoid ROS (Reactive oxygen species) generation that is associated with redox active metal catalysis involving the metal ion chelation. The plant extract interfered with the formation of ferrous and ferrozine complex, thus depicting that the extract has some chelating activity and thus captures ferrous ion before ferrozine. IC_{50} of the extract for chelating activity was 635µg/mL as shown in Figure. 5. **Antimicrobial activity**

Extract has shown moderate zone of inhibition against *S. aureus*, *B. cereus*, *P. aeroginosa*, *E. coli* and *K. pneumoniae* and the results were given in table 1.

Table 1: Antimicrobial activity of hydroethanolic extract of *K. galanga* Linn.

Test organisms	Inhibition zone diameter (mm) *K. galanga* leaves		
	Hydroethanolic extract	Positive Control	Negative Control
B. cereus	0.0±0.64	18.0±1.0	0.0±0.0
E. coli	0.0±0.81	11.0±1.5	0.0±0.0
K. pneumoniae	19±0.32	21±0.57	0.0±0.0
P. aeroginosa	14±0.41	26±0.57	0.0±0.0
S. aureus	12±0.72	14.0±0.57	0.0±0.0

Values are expressed as mean ± standard deviation of the three replicates. Zone of inhibition not include the diameter of the well

Hemolytic activity

Hemolytic activity potrays a competent information on the interaction between molecules and the cells at cellular level. Haemolytic activity of any compounds is usually an indicator of cytotoxicity towards normal

cells in our body[38]. *In vitro* haemolytic assay using spectrophotometry provides an effective method for the measurement of hemolysis at quantitative level and also furnishes the effect of different concentrations of biomolecules on the human erythrocytes. Hemolytic activity was performed to check the toxic effect of the plant extract on human red blood cells. It is expressed in % and was carried out in triplicates. All the concentrations of the plant extract exhibited low hemolytic activity towards human RBC. Thus the plant extract was not toxic and considered safe on human erythrocytes (Figure. 6).

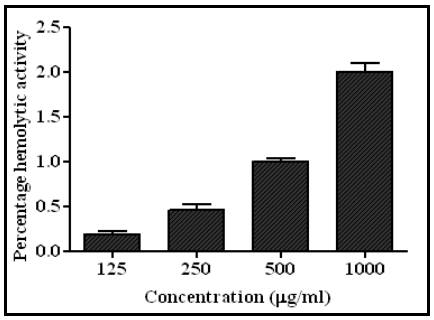

Figure.6 Hemolytic activity of the extract

FT-IR Analysis

FT-IR is a technique which is used to obtain an infrared spectrum of absorption, emission, or Raman scattering of a solid, liquid or gas[39-40]. An FT-IR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage as it measures intensity over a narrow range of wavelengths at a time. The results obtained in the study of the FT-IR spectrum represented that the extracts of *K. galanga* leaves contain a variety of phytochemical compounds, which can effectively prevent free radical mediated cell damage by free radicals scavenging activity and thus can be used as a potent source of natural antioxidant compounds. The FT-IR analysis revealed the presence of various functional groups such as alcohols, phenols, carbonyls, aromatic amines, alkanes, alkynes, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides (Figure. 7).

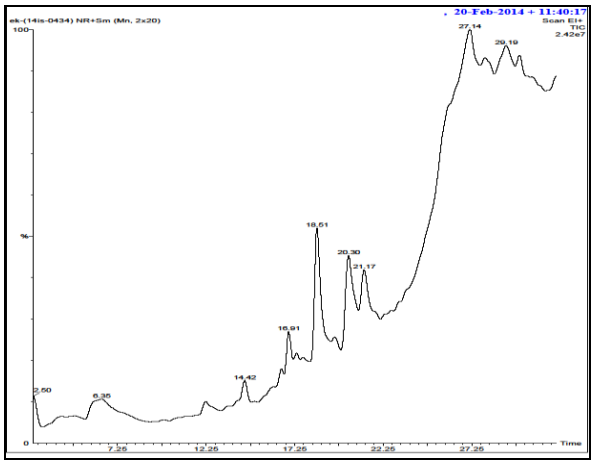

Figure.7 FT-IR analysis of the extract

HPLC analysis

HPLC analysis resulted in the identification of four phenolic compounds by comparing the sample retention times with the reference retention times which includes Genistein, Ferulic acid, Coumaric acid and Butein (Figure. 8). Results of HPLC analysis (Table 2) identified the presence of compounds Genistein, Ferulic acid, Coumaric acid and Butein. The identified phenolic compounds have also been reported previously on studies for antioxidant activities[41-42] and therefore conclude that this potent antioxidant property of the plant might be due to the presence of these phenolic compounds.

Table 2: HPLC based phenolic compounds identification of hydroethanolic extract of *K. galanga* Linn

Compound name	Sample retention time (min)	Reference retention time (min)	Wavelength (nm)
Genistein	78.852	79.8	250
Ferulic acid	25.39	25.8	320
Coumaric acid	23.819	23.3	320
Butein	79.148	79.2	320

Conclusion

K. galanga is a significant medicinal plant used to treat various diseases in folk medicine. This study concluded that the hydroethanolic extract of *K. galanga* leaves exhibited high antioxidant potential for the tests performed as well as shown moderate antibacterial and no hemolytic activities against human erythrocytes. HPLC analysis partially identified the presence of Genistein, Ferulic acid, Coumaric acid and Butein. Further, the bioactive compound has to be isolated from the plant and can be used as a safe remedy for free radical associated human diseases.

Acknowledgement

The authors would like to thank the Management and Staff of VIT University, Vellore, TN, India for providing the facilities and work ambience for the successful completion of our study.

References

1. Larson RA. The antioxidants of higher plants. Phytochemistry., 1988, 4; 969 -978.
2. Salahuddin A., Rahman M.S., Jasim U.C. Antimicrobial activities of seed extracts and true alkaloids of *Aegle marmelos*. Gre J Sci., 1998, 22; 77–81.
3. Techaprasan J., Klinbunga S., Ngamriabsakul C., Jenjittikul T. Genetic variation of *Kaempferia* (Zingiberaceae) in Thailand based on chloroplast DNA (psba-trnh and peta-psbj) sequences. Genet Mol Res., 2010, 9;1957-1973.
4. Scherer R., Godoy HT. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1- picrylhydrazyl method. Food Chemi., 2009, 112; 654-658.
5. Ou B., Huang D., Hampsch-Woodill M., Flanagan JA., Deemer EK. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (orac) and ferric reducing antioxidant power (frap) assays: A comparative study. J Agric Food Chem., 2002, 50; 3122-3128.
6. Shekhawat NS., Jain HC., Aryah C. Accumulation of aromatic amino acids, the precursors of auxin and phenols in pearl millet infected with *Sclerospora graminicola*. Comparative physiology & ecology., 1980, 5; 39-42.
7. Velioglu YS., Mazza G., Gao L., Oomah BD. Antioxidant activity and total phenolics in selected fruits,vegetables, and grain products. J Agric Food Chem., 1998, 46; 4113-4117.
8. Halliwell B., Gutteridge JMC., Free radicals in biology and Medicine. 3rd ed. Oxford university Press, New york, USA, 1999, 617-783.
9. Hertog MGL., Hollman PCH., Katan MB. Content of potentially anti-carcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric Food Chem., 1992, 40; 2379-2383.
10. Chu DM., Miles H., Toney D., Ngyuen C., Marciano-Cabral F. Amebicidal activity of plant extracts from Southeast Asia on *Acanthamoeba* spp. Parasit Res., 1998, 84; 746-752
11. Koh HL. Guide to Medicinal Plants: An Illustrated Scientific and Medicinal Approach. SGP. World Scientific., 2009.
12. Doreswamy R., Darshan S. Patented Anti-inflammatory Plant Drug Development from traditional medicine. Phytotherapy Research., 2004, 18; 343–357.
13. Peter KV., Cambridge GBR. Handbook of Herbs and Spices. Wood head Publishing Limited., 2004.
14. Yu JG., Yu DL., Zhang S., Luo XZ., Sun L. Studies on the chemical constituents of *Kaempferia marginata*. Acta Pharm Sin., 2000, 35; 760-763.
15. Chithra M., Martin KP., Sunandakumari C., Madhusoodanan PV. Protocol for rapid propagation and to overcome delayed rhizome formation in field established in vitro derived plantlets of *Kaempferia galanga* L. Sci. Hortic., 2005, 104; 113-120.

16. Kokate CK. Practical pharmacognosy, Kakatiya University, Warangal, A.P., India. 1994, 109-114.
17. Shimada K., Fujikawa K., Yahara K., Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem., 1992, 40; 945-948.
18. Jayaprakasha GK., Singh KP and Sakariah KK. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. Food Chem., 2001, 73; 285- 290.
19. Dinis TC., Madeira VM., Almeida L.M. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radicalscavengers. Arch Biochem Biophys., 1994, 315; 161-169.
20. Mandal TK., Chatterjee SN. Ultraviolet- and Sunlight-induced Lipid Peroxidation in Liposomal Membrane. Radiat Res., 1980, 83; 290-292.
21. Kumar G., Karthik L., Rao KVB. Antibacterial activity of aqueous extract of Calotropis gigantea leaves – an in vitro study. Int J Pharm Sci Rev Res., 2010, 4; 141-144.
22. Yang ZG., Sun HX., Fang W.H. Haemolytic activities and adjuvant effect of Astragalus membranaceus saponins (AMS) on the immune responses to ovalbumin in mice. Vaccine., 2005, 23; 5196-5203.
23. In-Ho Choi., Ju-Yong Park., Sang-Chul Shin., Il-Kwon Park. Nematicidal activity of medicinal plant extracts and two cinnamates isolated from *Kaempferia galanga* L. (Proh Hom) against the pine wood nematode, *Bursaphelenchus xylophilus*. Nematology., 2006, 8; 359-365.
24. Sakakibara H., Honda Y., Nakagawa S., Ashida H., Kanazawa K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. J Agric Food Chem., 2003, 51; 571-581.
25. Surveswaran S., Yi-Zhong Cai, Corkel H., Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry., 2007, 102; 938–953.
26. Chan EWC., Lim YY., Wong LF., Lianto FS., Wong SK., Lim KK., Joe CE., Lim TY. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species, Food Chemistry., 2008, 109; 477–483.
27. Krishnaiah D., Sarbatly R., Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev., 2007, 1; 97-104.
28. Mahato SB., Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry., 1997, 44;1185-1236.
29. Kappers IF., Aharoni A., van Herpen TW., Luckerhoff LL., Dicke M. Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. Science., 2005, 309 ; 2070-2072.
30. Hérouart D., Sangwan RS., Fliniaux MA., Sangwan-Norreel B.S. Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of *Datura innoxia*. Planta Med., 1988, 54; 14-17.
31. Scalbert A., Johnson IT., Saltmarsh M. Polyphenols: Antioxidants and beyond. Am J Clin Nutr., 2005, 81; 215–217.
32. Blokhina O., Virolainen E., Fagerstedt KV. Antioxidants,oxidative damage and oxygen deprivation stress: a review. Ann Bot., 2003, 91; 179–194.
33. Halliwell B. Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action. Free Radical Biol Med., 1989, 7; 645–651.
34. Evans P., Halliwell B. Micronutrients: oxidant/antioxidant status. Br J Nutr., 2001, 85; 67–74.
35. Kumar G., Karthik L., Rao KVB. A review on pharmacological and phytochemical profile of *Calotropis gigantea* Linn. Pharmacologyonline., 2011, 1;1-8.
36. Huang DJ., Ou BX., Prior R.L. The chemistry behind antioxidant capacity assays. J Agric Food Chem., 2005, 53; 1841-1856.
37. Halliwell B., Chirico S. Lipid Per-oxidation: its mechanism, measurement and significance. Am J clin Nutr., 1993, 57; 715-725.
38. Frankel EN. Recent advances in lipid oxidation. J Sci Food Agric., 1991, 54; 495-511.
39. Da Silva E., Shahgaldian P., Coleman AW. Haemolytic properties of some water-soluble para-sulphonato-calix-[n]-arenes. Int J Pharm., 2004, 273; 57-62.
40. Othman R., Ibrahim H., Mohd MA., Mustafa MR., Awang K. Bioassay-guided isolation of a vasorelaxant active compound from *Kaempferia galanga* L. Phytomedicine., 2006, 13; 61-66.
41. Rahman MM., Amin MN., Ahamed T., Ali MR., Habib A. Efficient plant regeneration through somatic embryogenesis from leaf base-derived callus of *Kaempferia galanga* L. Asian J Plant Sci., 2004, 3; 675-678.
42. Ian R., Ivor ED., Jennifer K.M. The antioxidant activity of genistein in vitro. J Nutr Biochem., 1995, 6; 481-485.

*****