# PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *KAEMPFERIA GALANGA* L.

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## Abstract

The medicinal plant *Kaempferia galanga* L.is locally known as kun-sar-gamone belonging to the family Zingiberaceae collected from ka-wa Township, Bago Region. Preliminary phytochemical tests, physicochemical properties and elemental analysis were carried out by using the powdered samples of the rhizomes. According to the physicochemical properties, the samples were more soluble in water than the other solvents. In the result of elemental analysis, the concentration of arsenic was found to be 0.00009%. The concentration of elements were studied by using Atomic Absorption Spectrometer (AAS) at Universities Research Centre (URC). In addition, nutritional values of the rhizomes were examined at the Food Industries Development Supporting Laboratory (FIDSL). Fats, fibers, proteins and carbohydrates were observed as nutritional contents. High content of carbohydrate was also found. Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L. were investigated at Pharmaceutical Research Department (PRD) by using agar-well diffusion method with nine pathogenic microorganisms. Ethylacetate extract showed that most significant activity against *E.coli* and *Proteus mirabilis*.

**Keywords**: *Kaempferia galanga* L., Qualitative and Quantitative analysis, Antimicrobial Activities.

## Introduction

The Zingiberaceaefamily occur mainly in the tropics and subtropics. Asia and consists of over 40 genera and about 1,000 species (Dassanayake, 1983).An indigenous plant, *Kaempferia galanga* L. belongs to family Zingiberaceae. *Kaempferia galanga L.*, is known as “Kun-sar, gamone” in Myanmar, “galangal (or) “*Kaempferia”* in English, “Shajiang” in China, “Chekur” in Malaysia, “Chandramula” in India and “Prohom in Thailand.

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This family is rhizomatous herbs with simple and distichous leave. In Peninsular Malaysia and Indonesia the leaves and rhizomes are chewed as an expectorant for coughs and sore throat or pounded and used in poultices or lotions, they are often used as an ingredient of children's medicines and tonics (De Padua *et al*., 1999).

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are chemical compounds that occur naturally in the medicinal plants (phyto means "plant" in Greek), leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds(Wadood *et al*., 2013).

Characterization of physicochemical properties attained strong interest in the pharmaceutical research area and is now a standard method. It is one of the key challenges to develop a pharmaceutical active ingredient into a drug, which combines biological activity with an appropriate physiochemical profile. Poor solubility in aqueous media is one of the major hurdles in the drug development process (Kerns *et al*., 2008).

Nutritive value of plant has its own importance. Carbohydrates fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively a smaller but never a less important part. The rhizome is rich in essential oil and is being used for the treatment of cold, headache, expectorant, diuretic, stomachic, coughs and asthma (Rajendra *et al*., 2011).

The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against

drug resistant microbial pathogen. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone and Boxter, 1995).

The aim of the research work is to explore the Myanmar medicinal plants and to promote the Myanmar traditional medicine scientifically. To achieve the aim, the objectives are to verify both vegetative and reproductive part of the plant, to perform the qualitative and quantitative analysis of rhizomes, to analyze the nutritional values, to find out the antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.

## Materials and Methods

### Morphological study of *Kaempferia galanga* L.

### Collection and identification

The plant specimens of *Kaempferia galanga* L. were collected from Ka-wa Township, Bago Region during the flowering and fruiting periods from June to October, in the year 2013. For morphological study, the specimens were measured, described in detail and identified with the help of available literatures (Hooker, 1894; Backer, 1968; Dassanayake, 1983; Wu Delin and Kai Larsen; 2000). The specimens such as habit, leaves, inflorescences and flowers were recorded with the photographs. Herbarium specimens were prepared and kept in the Herbarium, Department of Botany, University of Yangon.

### Qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

Preliminary phytochemical investigation on rhizomes from *Kaempferia galanga* L. was carried out to examine the plant constituents. The powdered rhizomes of *Kaempferia galanga* L. was tested qualitatively for the presence or absence of alkaloid, α-amino acid, carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin,

flavonoid, steroid and terpenoid. According to the methods of Marini Bettolo *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002), the investigation of phytochemcial studies was applied. The results were as shown in Figure (1) and Table (1).

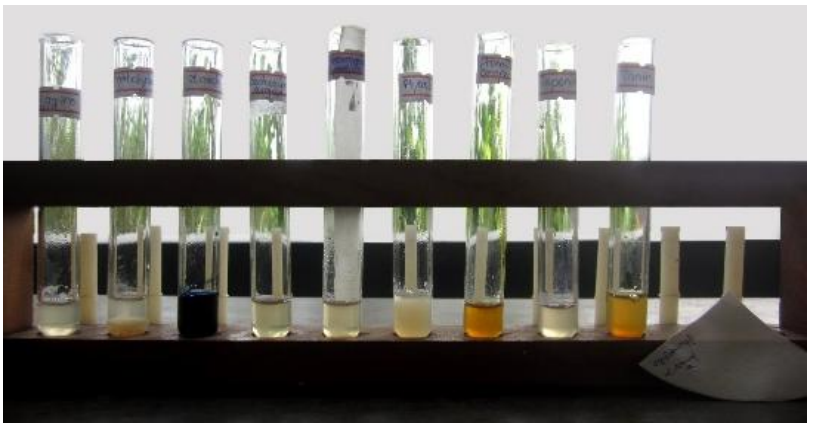


**Figure 1.** Phytochemical Test

**Quantitative analysis ofpowdered rhizomes from *Kaempferia galanga* L.**

In the quantitative analysis, moisture content, total ash, acid soluble ash, water soluble ash and various solvents such as ethanol, petroleum ether, methanol, ethyl acetate, chloroform, acetone and distilled water soluble contents were carried out according to the method of British Pharmacopoeia (1968).

**Elemental analysis of powdered rhizomes from *Kaempferia galanga* L. by using Energy Dispersive X-Ray Fluorescence spectrophotometer (EDXRF)**

The relative concentration of elements inpowdered rhizomes of *Kaempferia galanga* L.were analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrophotometer, Shimadzu Co. Ltd, Japan, at the

Physics Department, University of Mandalay. The parameters of each part of the spectrophotometer are as follows:

| | |
|---|---|
| Detector Type | Si (Li) detector |
| Liquid $N_2$ Supply | Only during measurement |
| Detection area | 10 $mm^2$ |
| Resolution | Less than 155eV (MnKa 1500Hz) |

The EDX-700 Shimadzu spectrometer can detect a wide range of the elements from aluminium (Al) to uranium (U). The required data can be produced in a few minutes and it has a high degree of resolution for the spectrum evaluation. The powdered sample was pressed into pellet by a hydraulic press of 4 tons. The pellet was used in the EDX-700. Shimadzu Spectrophotometer which produced the X-ray spectrum, consisting of the respective elements. The spectrum evaluation was carried out by the use of the built in elemental analysis software.

**Detection of heavy metals by using Atomic Absorption Spectrophotometric (AAS) analysis of powdered rhizomes from *Kaempferia galanga* L.**

Some minerals namely sodium, calcium, potassium, copper, chromium, magnesium, iron, zinc and manganese were quantitatively analyzed by Perkin and Elmer Analyst 800 spectrophotometer. Ten grams of powdered sample was placed in a weighed crucible and heated in a Muffled furnace at 500°C to achieve completely ash. About 0.5 g of ash was filtered with 300 meshes and digested in 5 ml of concentrated hydrochloric acid. The solution was evaporated overnight to dryness in air and the residue was leached in a water bath treated with 5 ml of hydrochloric acid mixture at a temperature of about 70°C for 30 min. The solution was stirred by using vortex mixer. These solutions were decanted and the clear solution was made up to 100 ml with deionized water. Ten ml of the resultant solution was

pipette accurately and made up to 100 ml with deionized water again the solutions were stand overnight and then were aspirated on an atomic absorption a spectrophotometer. Atomic Absorption Spectrophotometer measured the atomic vapor produced from a sample solution by light from a Hollow Cathode Lamp (HCL) and Electrode less Discharge Lamp (EDL) that emitted characteristic light wavelength of an element. The light was absorbed by the atoms of the element present in the flame. The degree of absorption was measured by photomultiplier tube.

**Nutritional values of powdered rhizomes from *Kaempferia galanga* L.**

The rhizomes of *Kaempferia galanga* L. were evaluated for its nutritive value at Food Industries Development Supporting Laboratory (FIDSL), Yangon. The nutritional value had been undertaken according to the method of Association of Official Analytical Chemists (AOAC) (Horwitz, 1980).

**Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.**

**Apparatus**

Autoclave, beaker, bottle, conical flask, clean bench, cotton wool, hot air sterilizer, loops, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipette and water bath.

**Microorganisms**

The solvent extracts of rhizomes were tested against nine pathogenic microorganisms by using agar-well diffusion method. In the test microorganisms *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae and Proteus mirabilis* were included. The test was conducted at the Pharmaceutical Research Department (PRD).

## Procedure for antimicrobial activity

The study of antimicrobial activities was performed by agar-well diffusion method and nutrient agar was prepared according to the method of Cruickshank (1975). Nutrient agar was boiled and 20 - 25 ml of the medium was poured into each test tube and plugged with cotton wool and sterilized at 121 °C for 15 minutes in an autoclave. Then, the tubes were cooled down to 30-35°C and the content was poured into sterilized petridishes and 0.1 - 0.2 ml of test organism was also added into the dishes. The agar was allowed to set for 2 - 3 hours. Then, 10 mm plate agar-well was made with the help of sterilized agar-well borer. After that, about 0.2 ml of sample was introduced into the agar-well and incubated at 37 °C for 24 hours. The inhibition zone appeared around the agar-well, indicating the presence of antimicrobial activity. The diameter of the inhibition zones were measured with the help of transparent ruler, at the diameter zone of inhibition including the agar well.

**Table 1.** Types of microorganisms and their diseases

| No | Type of microorganism | Diseases |
|---|---|---|
| 1. | *Bacillus subtilis* | Diarrhoea and food poisoning |
| 2. | *Staphylococcus aureus* | Pneumonia, skin infections and food poisoning |
| 3. | *Pseudomonas aeruginosa* | Urinary tract infection, gastrointestinal infection, inflammation |
| 4. | *Bacillus pumalis* | Food poisoning and eye infections |
| 5. | *Candida albicans* | Vaginal infection and skin infection |
| 6. | *Escherichia coli* | Urinary tract infections, diarrhoea and dysentery |
| 7. | *Vibrio cholera* | Vomiting, diarrhoea |
| 8. | *Klebsiella pneumonia* | Pneumonia, urinary tract infections |
| 9. | *Proteus mirabilis* | Wound infections, urinary tract infection and pneumonia |

(Cruickshank, 1975)

## Results

### Morphological characters of *Kaempferia galanga* L.

Scientific name - *Kaempferia galanga* L.

Myanmar name - Kun-sar-gamone

English name - Sand ginger, Aromatic ginger

Family - Zingiberaceae

Herbs with aromatic rhizome, rhizomes yellowish white inside, fragrant. Leaves opposite and distichous, simple, the lamina broadly elliptic to slightly orbicular, 12.0-15.5cm long and 10.0-13.1cm wide, the bases cuneate, the margins entire, the tips acuminate, both surfaces glabrous; shortly petioles; leaf-sheath open,10.0 -23.0 cm long and 0.35 - 0.5 cm wide. Inflorescence terminal, compact spike; bracts lanceolate, 5.8-6.0 cm long and about 1.0 cm wide. Flower white with violet center, 5.6- 5.8 cm long and 2.6-2.8 cm wide, complete, bisexual, irregular, zygomorphic, trimerous, epigynous; sepals (3), synsepalous, tubular, 2.0-2.3 cm long and about 0.3 cm wide, spathaceous splitting, white; petals (3), synpetalous, tubular, the tubes 3.6-3.8 cm long and about 0.3 cm wide, the lobes 1.6-1.8 cm long and 0.25-0.35 cm wide, white; stamens 1+ $(2)^{st}$+$2^{st}$, epipetalous, 1 fertile stamen4.5mm long and 5.0mm wide , 2 - outer staminodes fused to form a labellum, 17.0mm long and 18.0mm wide, white with violet center and 2- inner lateral petaloid free staminodes, 12.0 mm long and 8.0 mm wide , filaments grooved, exserted, anthers dithecous, introrse, dorsifixed, longitudinal dehiscence; ovary inferior, ovoid, 2.5 mm long and 2.0 mm wide, tricarpellary, syncarpous, trilocular, axile placentation, the style long and slender, 37.0mm long and 0.5 mm wide the stigma capitate as shown in Figures (2 to 18).Fruits and seeds not seen. Flowering and fruiting time is June to October.

## Morphological characters of *Kaempferia galanga* L.

**Figure 2.** Plants in natural habit

**Figure 3.** Close-up view of plant

**Figure 4.** Habit

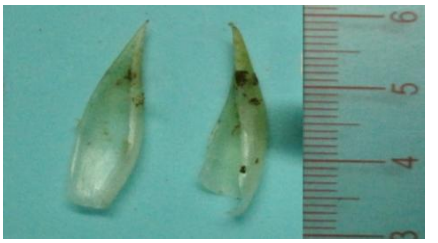
**Figure 5.** Rhizome

**Figure 6.** T.S of rhizome

**Figure 7.** Ventral view of leaves

**Figure 8.** Dorsal view of leaves

**Figure 9.** Bract with flower



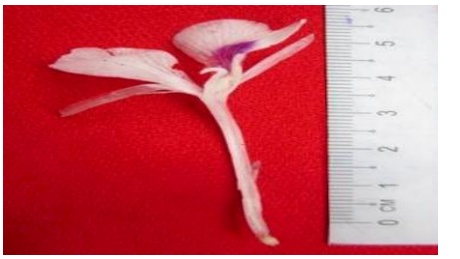


**Figure 10.** Bract

**Figure 12**. Calyx

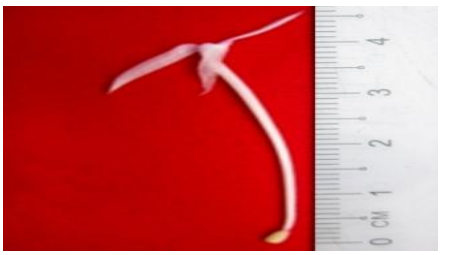
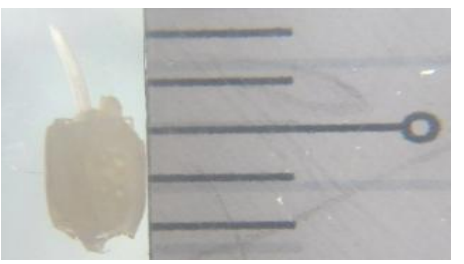


**Figure 13.** Corolla

**Figure 14.** Corolla with petaloid staminodes and fertile stamen

**Figure 15.** Pistil



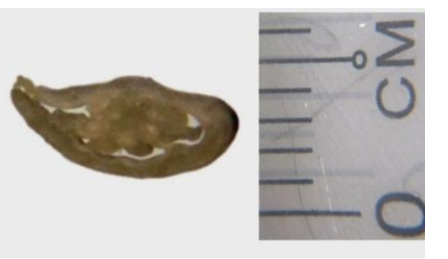


**Figure 16.** L.S of ovary

**Figure 17.** T.S of ovary

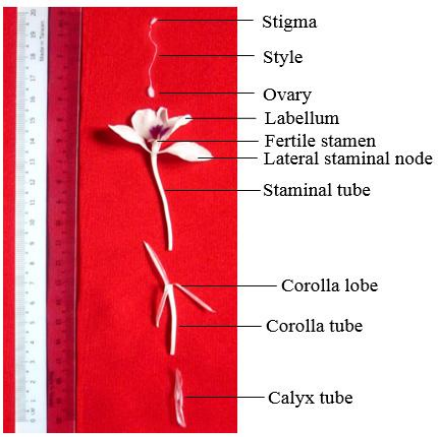



**Figure 18.** Floral parts

## Qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

In preliminary phytochemical test, the presence or absence of alkaloid, α-amino acids**,** carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid were observed in the rhizomes. α-amino acids and cyanogenic glycoside were absent. The results were shown in Table (2).

**Table 2.** The qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

| No | Test | Extract | Test Reagents | Observation | Result |
|---|---|---|---|---|---|
| 1 | Alkaloid | 1%HCL | (1)Mayer's Reagent<br>(2)Wagner's Reagent<br>(3)Dragendroff's Reagent | White ppt<br>Brown ppt<br>Orange ppt | +<br>+<br>+ |
| 2 | α-amino acids | $H_2O$ | Ninhydrin solution | No change in colour | - |

| | | | | | |
|---|---|---|---|---|---|
| 3 | Carbohydrate | $H_2O$ | 10% α-naphthol+conc-$H_2SO_4$ | Red ring | + |
| 4 | Starch | $H_2O$ | $I_2KI$ solution | Blue color | +++ |

| No | Test | Extract | Test Reagents | Observation | Result |
|---|---|---|---|---|---|
| 5 | Reducing sugar | $H_2O$ | Benedict's solution | Brick red ppts | + |
| 6 | Cyanogenic glycoside | $H_2O$ | (1)Conc-$H_2SO_4$ acid<br>(2)Sodium picrate paper | No change in color | - |
| 7 | Glycoside | $H_2O$ | 10%lead acetate solution | White ppts | + |
| 8 | Phenolic compound | $H_2O$ | Ferric chloride | Deep blue color | + |
| 9 | Saponin | $H_2O$ | Distilled water | Frothing | + |
| 10 | Tanin | $H_2O$ | Ferric chloride | Deep blue color | + |
| 11 | Flavonoid | EtOH | (1)Mg turning<br>(2)Conc HCL acid | Pink color | + |
| 12 | Steroid | P.E | Acetic anhydride+conc- $H_2SO_4$ | Blue green color | + |
| 13 | Terpenoid | P.E | Acetic anhydride+conc- $H_2SO_4$ | Deep pink color | + |

(+) present (-) absent ppt (precipitate)

**Quantitative analysis of powdered rhizomes from *Kaempferia galanga* L.**

In physicochemical properties moisture content, total ash, acid insoluble, water soluble ash and solubility in different solvents of rhizomes of *Kaempferia galanga* L. were observed. According to this result, the solubility of the powdered rhizomes in water soluble matter content was to be highest and moderately soluble in methanol as shown in Table (3).

**Table 3.** The quantitative analysis of powdered rhizomes from *Kaempferia galanga L.*

| No | Physicochemical characters | Average(%) |
|---|---|---|
| 1 | Moisture content | 7.57 |
| 2 | Total ash content | 8.01 |
| 3 | Water soluble ash content | 0.4 |
| 4 | Acid insoluble ash content | 0.7 |
| 5 | Ethanol soluble matter content | 30.0 |
| 6 | Methanol soluble matter content | 10.0 |
| 7 | Pet-ether soluble matter content | 20.0 |
| 8 | Ethyl-acetate soluble matter content | 20.0 |
| 9 | Chloroform soluble matter content | 25.0 |
| 10 | Acetone soluble matter content | 30.0 |
| 11 | Water soluble matter content | **35.0** |

**Table 4.** Elemental analysis of powdered rhizomes from *Kaempferia galanga* L.

| No | Symbol | Element | Concentration value rhizomes (%) |
|---|---|---|---|
| 1. | Cd | Cadmium | 0.0006 |
| 2. | P | Phosphorus | 0.2564 |
| 3. | Ca | Calcium | 0.1645 |
| 4. | K | Potassium | 2.616 |
| 5. | Cu | Copper | 0.00051 |
| 6. | Fe | Iron | 0.07039 |
| 7. | Pb | Lead | 0.00009 |
| 8. | Mn | Manganese | 0.02189 |
| 9. | S | Sulfur | 0.04701 |
| 10. | Zn | Zinc | 0.00266 |
| 11. | Hg | Mercury | 0.00014 |
| 12. | Cr | Chromium | 0.00084 |
| 13. | As | Arsenic | 0.00009 |

**Elemental analysis of powdered rhizomes from *Kaempferia galanga* L. by using Energy Dispersive X-ray Fluorescence (EDXRF)**

In order to determine the heavy toxic metals and macronutrient elements in plant samples, qualitative elemental analysis was performed by EDXRF method at the Universitie's Research Centre, University of Yangon. Pellets of samples (2.5 cm diameter) were first made by using a pellet making machine. X-ray spectrometer permits simultaneous analysis of light element to heavy element (Griken *et .al*., 1986). Energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-700) can analyze the elements from AL to U under vacuum condition. X-ray fluorescence uses X-rays to excite an unknown sample. The individual elements comparising in the sample remit their own characteristic X-rays. They are detected by using semiconductor detector [Si (Li)] that permits simultaneous analysis of multi elements within the sample. In this way, EDX-700 spectrometer determines the elements that are present in the sample. It can perform two kinds of quantitative analysis: the Fundamental Parameter (FP) Method and the Calibration Curve Method. In the (FP) method, theoretical results can be calculated even when standard sample are not available. It can be applied to most samples but the accuracy must be checked in advance. In the calibration curve method, experimental results can be obtained by using standard sample. Although Limited sample can be applied, the accuracy is high. In the present study, the FP method was applied for the elemental analysis. The results were reported and discussed in Figure (19) and Table (4).

SPECTRO X-LabPro — Job Number: XRF2014

Preset Sample Data

| | | | |
|---|---|---|---|
| Sample Name | **Rhizome** | Dilution Material | M_HWC |
| Description | | Sample Mass (g) | 5.0000 |
| Method | FP-Pellets-121997ne1 | Dilution Mass (g) | 1.0000 |
| Job Number | XRF2014 | Dilution Factor | 0.8333 |
| Sample Type | Pressed tablet, 32 mm | Sample rotation | No |
| Sample State | Pellet_32 | Date of Receipt | 01/10/2014 12:57:41 |
| Sample Status | A X X A X X X A | Date of Evaluation | 01/10/2014 12:58:06 |

Results

The error is the statistical error with 1 sigma confidence interval

| Z | Symbol | Element | Norm. Int. | Concentration | Abs. Error |
|---|---|---|---|---|---|
| 13 | Al | Aluminum | 132.0450 | 0.1626 % | 0.0028 % |
| 14 | Si | Silicon | 387.7035 | 0.2459 % | 0.0015 % |
| 15 | P | Phosphorus | 1236.4361 | 0.2564 % | 0.0007 % |
| 16 | S | Sulfur | 992.6904 | 0.04701 % | 0.00011 % |
| 17 | Cl | Chlorine | 2302.1015 | 0.2938 % | 0.0006 % |
| 19 | K | Potassium | 1683.8357 | 2.616 % | 0.004 % |
| 20 | Ca | Calcium | 155.1122 | 0.1645 % | 0.0010 % |
| 22 | Ti | Titanium | 14.9347 | 0.01182 % | 0.00034 % |
| 23 | V | Vanadium | 5.8083 | 0.00337 % | 0.00024 % |
| 24 | Cr | Chromium | 8.6737 | 0.00084 % | 0.00005 % |
| 25 | Mn | Manganese | 141.7020 | 0.02189 % | 0.00018 % |
| 26 | Fe | Iron | 757.0658 | 0.07039 % | 0.00024 % |
| 27 | Co | Cobalt | 0.9702 | < 0.00030 % | (0.0) % |
| 28 | Ni | Nickel | 3.4475 | 0.00007 % | 0.00001 % |
| 29 | Cu | Copper | 16.9398 | 0.00051 % | 0.00002 % |
| 30 | Zn | Zinc | 118.3588 | 0.00266 % | 0.00002 % |
| 31 | Ga | Gallium | 0.3105 | < 0.00005 % | (0.0) % |
| 32 | Ge | Germanium | 0.0000 | < 0.00005 % | (0.0) % |
| 33 | As | Arsenic | 9.4628 | 0.00009 % | 0.00001 % |
| 34 | Se | Selenium | 5.3297 | 0.00005 % | 0.00001 % |
| 35 | Br | Bromine | 99.1746 | 0.00081 % | 0.00001 % |
| 37 | Rb | Rubidium | 488.7517 | 0.00237 % | 0.00001 % |
| 38 | Sr | Strontium | 91.3289 | 0.00040 % | 0.00001 % |
| 39 | Y | Yttrium | 23.8606 | 0.00010 % | 0.00001 % |
| 40 | Zr | Zirconium | 0.0000 | < 0.00010 % | (0.0) % |
| 41 | Nb | Niobium | 0.0000 | < 0.00010 % | (0.0) % |
| 42 | Mo | Molybdenum | 1.8134 | 0.00007 % | 0.00002 % |
| 47 | Ag | Silver | 0.0000 | < 0.00020 % | (0.0) % |
| 48 | Cd | Cadmium | 2.6547 | 0.00006 % | 0.00002 % |
| 50 | Sn | Tin | 6.8609 | 0.00045 % | 0.00006 % |
| 51 | Sb | Antimony | 4.6696 | 0.00037 % | 0.00007 % |
| 52 | Te | Tellurium | 2.6345 | < 0.00030 % | (0.0) % |
| 53 | I | Iodine | 3.3498 | 0.00021 % | 0.00008 % |
| 55 | Cs | Cesium | 0.0000 | < 0.00040 % | (0.0) % |
| 56 | Ba | Barium | 5.2036 | 0.00132 % | 0.00050 % |
| 57 | La | Lanthanum | 0.0000 | < 0.00020 % | (0.0) % |
| 58 | Ce | Cerium | 0.0000 | < 0.00020 % | (0.0) % |
| 72 | Hf | Hafnium | 9.9867 | 0.00057 % | 0.00003 % |
| 73 | Ta | Tantalum | 6.6298 | 0.00032 % | 0.00003 % |
| 74 | W | Tungsten | 0.0000 | < 0.00010 % | (0.0) % |
| 80 | Hg | Mercury | 6.9467 | 0.00014 % | 0.00001 % |
| 81 | Tl | Thallium | 4.6311 | 0.00006 % | 0.00001 % |
| 82 | Pb | Lead | 4.9869 | 0.00009 % | 0.00002 % |
| 83 | Bi | Bismuth | 5.6595 | 0.00011 % | 0.00001 % |
| 90 | Th | Thorium | 1.0414 | < 0.00010 % | (0.0) % |
| 92 | U | Uranium | 9.7474 | < 0.00010 % | (0.0) % |
| | | Sum of concentration | | 3.91 % | |

**Figure 19.** EDXRF data of relative elements contents of the powdered rhizomes from *Kaempferia galanga* L.

**Detection of heavy metals by using Atomic Absorption Spectrometric (AAS) analysis of powdered rhizomes from *Kaempferia galanga* L.**

The content of heavy metals were analysed by using AAS, measured in the unit of mg/L. According to AAS, arsenic, cromium, lead and mercury were not found detected. The results were described in details as shown in Table (5).

**Table 5.** Elemental analysis of powdered rhizomes from *Kaempferia galanga* L.by using AAS

| No | Elements | PPM |
|---|---|---|
| 1 | Arsenic(As) | ND |
| 2 | Cadmium(Cd) | 0.007 |
| 3 | Cromium(Cr) | ND |
| 4 | Lead(Pb) | ND |
| 5 | Mercury (Hg) | ND |

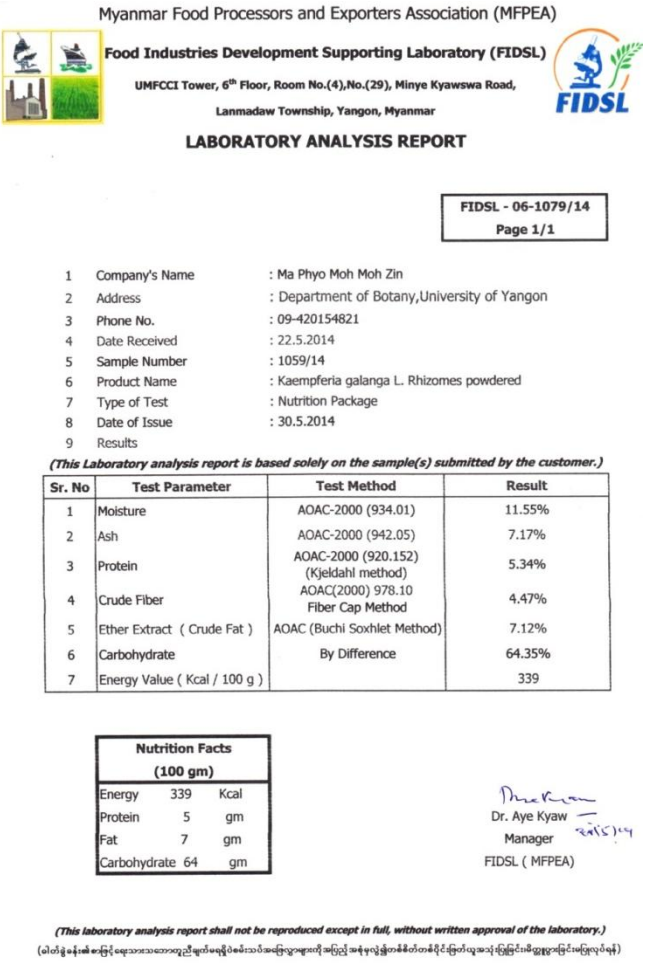
N.D= Not Detected

**Nutritional values of powdered rhizomes from *Kaempferia galanga* L.**

The nutritional contents such as protein, fiber, fat, carbohydrate of rhizomes from *Kaempferia galanga* L. was analysed at Food Industries Developed Supporting Laboratory (FIDSL). Among them, carbohydrate was found to the highest content. The results were shown in Figure (20) and Table (6).

**Table 6.** Nutritional value of powdered rhizomes from *Kaempferia galanga* L.

| No | Type of Nutrients | Content(gm) |
|---|---|---|
| 1 | Protein | 5.34 |
| 2 | Fat | 7.12 |
| 3 | Carbohydrate | **64.35** |
| 4 | Energy Value (Kcal/100g) | 339 |



Myanmar Food Processors and Exporters Association (MFPEA)

Food Industries Development Supporting Laboratory (FIDSL)

UMFCCI Tower, 6th Floor, Room No.(4),No.(29), Minye Kyawswa Road,
Lanmadaw Township, Yangon, Myanmar

FIDSL

**LABORATORY ANALYSIS REPORT**

**FIDSL - 06-1079/14**
**Page 1/1**

| | | |
|---|---|---|
| 1 | Company's Name | : Ma Phyo Moh Moh Zin |
| 2 | Address | : Department of Botany,University of Yangon |
| 3 | Phone No. | : 09-420154821 |
| 4 | Date Received | : 22.5.2014 |
| 5 | Sample Number | : 1059/14 |
| 6 | Product Name | : Kaempferia galanga L. Rhizomes powdered |
| 7 | Type of Test | : Nutrition Package |
| 8 | Date of Issue | : 30.5.2014 |
| 9 | Results | |

***(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)***

| Sr. No | Test Parameter | Test Method | Result |
|---|---|---|---|
| 1 | Moisture | AOAC-2000 (934.01) | 11.55% |
| 2 | Ash | AOAC-2000 (942.05) | 7.17% |
| 3 | Protein | AOAC-2000 (920.152) (Kjeldahl method) | 5.34% |
| 4 | Crude Fiber | AOAC(2000) 978.10 Fiber Cap Method | 4.47% |
| 5 | Ether Extract ( Crude Fat ) | AOAC (Buchi Soxhlet Method) | 7.12% |
| 6 | Carbohydrate | By Difference | 64.35% |
| 7 | Energy Value ( Kcal / 100 g ) | | 339 |

| Nutrition Facts (100 gm) | | |
|---|---|---|
| Energy | 339 | Kcal |
| Protein | 5 | gm |
| Fat | 7 | gm |
| Carbohydrate | 64 | gm |

Dr. Aye Kyaw
Manager
FIDSL ( MFPEA)

***(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)***

**Figure 20.** Nutritional values of powdered rhizomes from *Kaempferia galanga L.*

## Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.

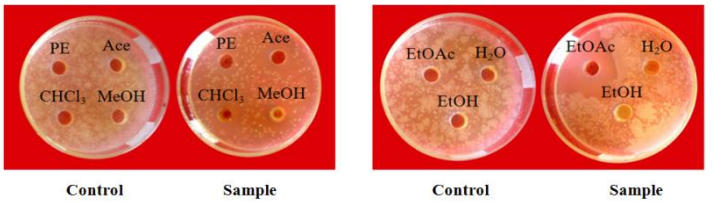
Antimicrobial activity was studied with 70% pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol, aqueous extract. Agar-well

diffusion method was used to determine the zone of inhibition of microbial growth at particular concentration of various extracts are as shown in Tables (7) and Figures (21 to 23).

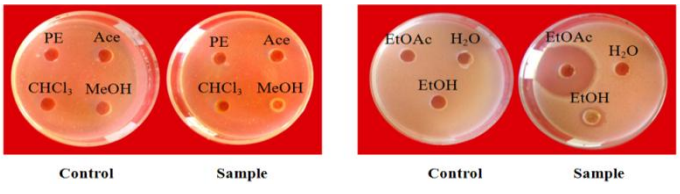
**Table 7.** Antimicrobial activity against nine test microorganisms by using various solvent extracts of rhizomes from *Kaempferia galanga* L.

| Extracts | Test Organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Bacillus subtilis* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Bacillus pumalis* | *Candida albicans* | *Escherichia coli* | *Vibrio cholerae* | *Klebsiella pneumoniae* | *Proteus mirabilis* |
| Pet-ether | 18mm | 18mm | 15mm | 11mm | - | - | - | - | 12mm |
| $CHCl_3$ | 16mm | 20mm | 13mm | 12mm | 11mm | - | - | - | 19mm |
| MeOH | 17mm | 20mm | 15mm | 14mm | 12mm | - | - | - | 30mm |
| Acetone | 20mm | - | 13mm | 13mm | 12mm | - | 16mm | - | 20mm |
| EtOAc | 35mm | 34mm | 36mm | 45mm | 36mm | 50mm | 45mm | - | 50mm |
| EtOH | - | 22mm | 15mm | 12mm | 12mm | 14mm | - | - | 22mm |
| $H_2O$ | - | - | - | - | - | - | - | - | - |

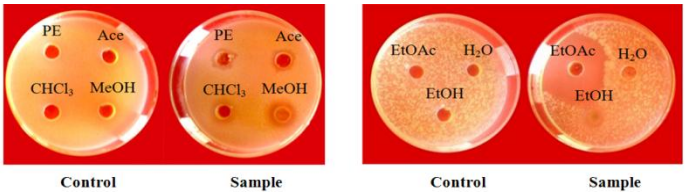
Agar well-10mm (-) Absent




***Bacillus subtilis***

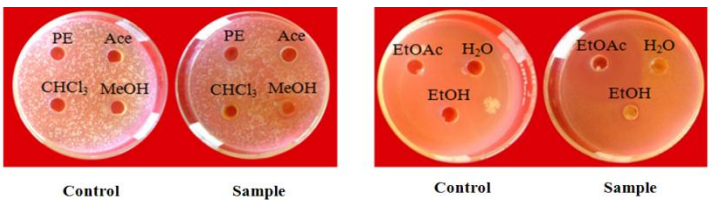



***Staphylococcus aureus***

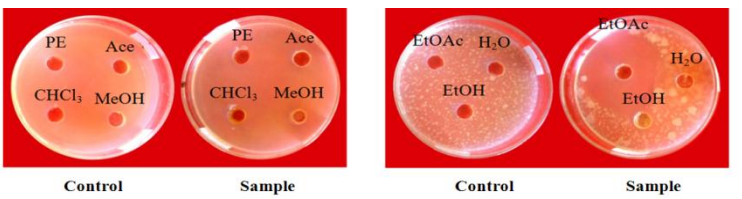



***Pseudomonas aeruginosa***

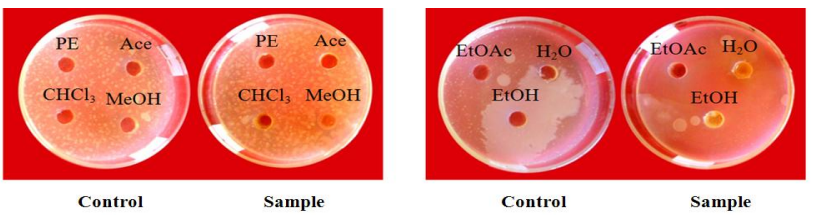
**Figure 21.** Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.




***Bacillus pumalis***




***Candida albicans***




***Escherichia coli***

**Figure 22.** Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.

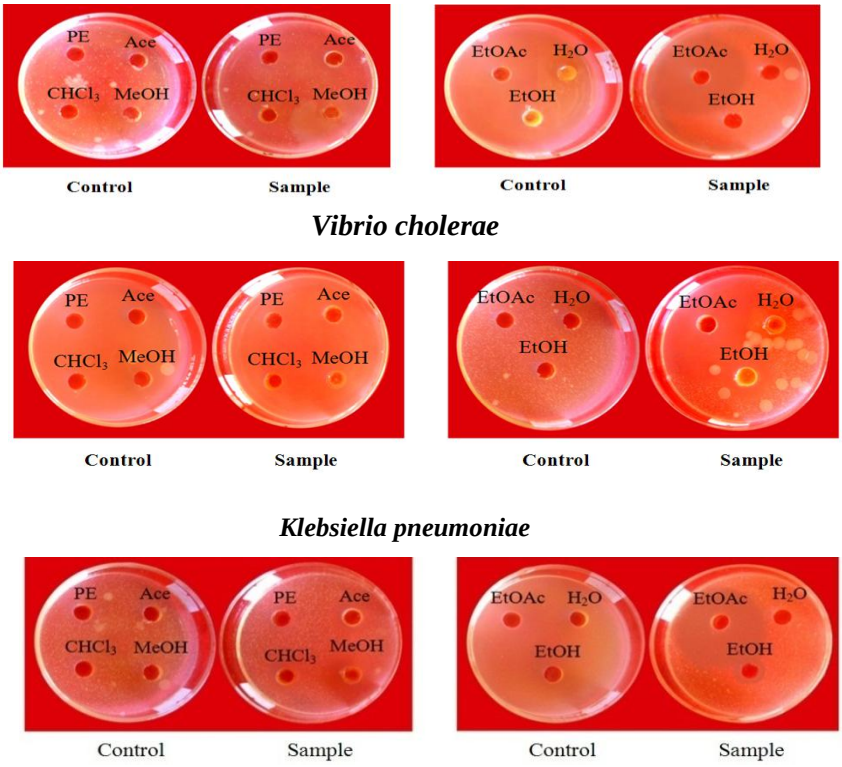



**Figure 23.** Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.

## Discussion and Conclusion

*Kaempferia galanga* L. (Kun-sa-gamone) is a medicinal plant belongs to the family Zingiberaceae.In the present research, the outstanding characters of *Kaempferia galanga* L. are rhizomatous herbs with yellowish white. Leaves are opposite and distichous and broadly elliptic lamina with shortly petiole and leaf-shealth. Inflorescence compactly spike, white with violet center flower. Sepals are (3), tubular, white and spathaceous splitting. Petals are(3),

tubular with reflexed lobes, white. Stamens are $1+(2^{st})+2^{st}$, epipetalous, one fertile stamens, white with violet center labellum, lateral petaloid staminodes. Ovary is tricarpellary, trilocular, axile placentation and stigma capitate. These characters were agreement with those given by Lawrence (1964), Ridley and Hutchinson (1967), Dassanayake (1983) and Jiang ke (2000).

In this research, alkaloid, carbohydrate, starch, reducing sugar, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid were detected. However α-amino acid and cyanogenic glycoside were not foundof rhizomes from *Kaempferia galanga* L. According to these results, *Kaempferia galanga* L. can be concluded as a plant rich in primary and secondary metabolites. In physicochemical examination, the percentage of soluble matters were calculated and found to contain the different yield contents. The powdered sample of *Kaempferia galanga* L. was mostly soluble in water. Experiments were carried out to find out moisture content, total ash, acid insoluble-ash, water soluble ash content and solubility matter in different solvents. The highly soluble solvent can be used for the extraction of active constituents.

Elemental analysis of the rhizomes were investigated by using EDXRF; Phosphorus (P), Potassium (K), Sulphur (S), Iron (Fe) and Calcium (Ca), were macroelements and Zinc (Zn), Copper (Cu), Molybdenum (Mo), Chlorine (Cl) and Nickel (Ni) were microelements. Among them, potassium was found to be the highest percentage. In this research, toxic elements, arsenic, chromium, lead, mercury were not found in rhizomes except cadmium according to Atomic Absorption Spectrometric Analysis. According to the result, nutritional contents of carbohydrate is high in the rhizomes of *Kaempferia galanga* L. carbohydrates are essential part of any diet. Carbohydrates provides the body with the energy it needs and are a good source of many vitamins and minerals (http://www.nutritional values, Human nutrition.com).

In antimicrobial activities of different solvents extracts were tested on nine pathogenic microorganisms by using agar-well diffusion method. Especially, ethyl-acetate extract was more effective than other solvents extracts but aqueous extract did not show antimicrobial activity. Antimicrobial activities of various solvents extracts did not effect on *Klebsiella pneumonia.* Kochuthressia *et.al*., (2012) revealed that various extracts such as ethanol, methanol, pet-ether, chloroform, aqueous extract showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Candida albicans* respectively.

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