Phytochemical and Biological Investigation of *Kaempferia galangal* Leaves

A project submitted by

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Dedicated to my parents for their selfless affection and dedication towards all my achievements and supporting me unconditionally at all the stages of the life.

Certification Statement

This is to certify that, this project titled 'Total Phenol Content Quantification, *in-vitro* Investigation of Antioxidant Activity, Cytotoxic and Antimicrobial Effect of the Methanol and Acetone Extract of *Kaempferia galangal* Leaves submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Mr. Imon Rahman, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where the ideas or writings has been used from others.

Signed,

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Countersigned by the supervisor

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Abstract

Kaempferia galangal is also known as aromatic ginger, resurrection lily, kencur or sand ginger, belongs to the ginger family which is a monocotyledonous plant. These plants are very famous for their medicinal use worldwide and especially in Indian regions. This plant is not only famous for their medicinal use but also for cooking and cosmetic purposes. In this particular study, in-vitro antioxidant activity of the methanol and acetone extract of *Kaempferia galangal* leaves was determined be DPPH (2, 2-diphenyl-1-picrylhydraziyl) free radical scavenging assay. Brine shrimp lethality test was performed to determine the cytotoxic effect and the LC_{50} value was calculated and has been compared to standard.

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Abbreviations

ACL - Acetone extract of leaves

ACR - Acetone extract of rhizome

ASA - Ascorbic Acid

BTH - Butyl-1- hydroxyl toluene

Ca - Calcium

CAE - Crude Acetone extract

CHF - Chloroform fraction

CME - Crude Methanol extract

DMSO - Dimethyl sulphoxide

DNA - Deoxyribonucleic acid

DPPH - 1, 1-diphenyl-2-picrylhydrazyl

FCR - Folin-Ciocalteu reagent

GAE - Gallic Acid Extract

HCl - Hydrochloric acid

HPLC - High Performance Liquid Chromatography

K - Potassium

Mg - Magnesium

Mn - Manganese

MEF - Methanol fraction

NaCl - Sodium Chloride

NaOH - Sodium Hydroxide

P - Phosphorus

PEF - Petroether fraction

ROS - Reactive Oxygen Species

S - Sulphur

SOD - Superoxide dismutase

STD - Standard Deviation

TPC - Total Phenol Content

VS - Vincristine Sulphate

WHO - World Health Organization

Zn - Zinc

Chapter One:

Introduction

1.1 Introduction

In between the plants there is a famous group of plants and that are medicinal plants which refers to the group of plants being practiced in Herbalism. The word herbalism came from the ‘herbilogy’ or we can also say ‘herbal medicine’. Medicinal plants serves in the medicinal purposes.

In the past, it was thought that plants without woods including shrubs are considered as medicinal plants. But the concept has changed now. In recent years all kind of plant parts may be referred as herbs and they are influencing the production of many products such as food, medicinal, cosmetic product etc.

From the Ancient period herbs or plants are influencing the study and use of medical science. Historically tribes such as Rome, Egypt, Iran, Africa and America had the tradition of using herbs or medicinal plants to get rid from the diseases for 4000 years at past. At the same time ongoing medicinal system such as Chinese medicine, Unani and Ayurveda were also being used at the same phase.

The use of medicinal system like Chinese medicine, Unani and Ayurveda has been continued and will be continued at a big range to minimize the damage being caused because of the human birth rate, less production and supply of medicinal compounds, expensive curing system, adverse effects of existing medicinal products and producing the compounds capable of facing and minimizing the upcoming threats in the history of human disease.

If we look at the past history of the medicinal plants or herbs using countries then we can see the India was one of the leading countries in that history because the forests of India were and still are rich with the varieties of plants including medicinal plants. At that period India alone found and used almost 8,000 medicinal plants to cure diseases. Basing on the plants there were so many ongoing medicinal system in India and between them Ayurveda and Unani were spread in a bigger range from past to present.

At present WHO (World Health Organization) said that only 20% of the people in the world does not depend on the herbs at all for their medicinal purpose and the rest depend on it in few

conditions in their disease curing period. It has been also said by WHO that more than twenty thousand of herbs or plants are qualified in showing their medicinal purpose.

Recent studies also show that people all over the world depends on the herbs or medicinal plants at most or fewer situations in their whole life. In the economically rich regions such as America, more than 20 percent people use herbs in their medicinal points. On the other hands economically medium ranger regions such as India and China's net drug compounds contain herbal components in a great range and that is more than 80 percent although these countries have a great contribution all over the world on medicinal purposes.

All over the world herbal medication system are being praised day and the use of the system is increasing day by day because of few specific reasons.

1. Herbs are known for having almost no side effect or the range is very low.
2. As it is believed human are made up of nature and herbs also directly come from nature so this two are synchronized in a really good manner.
3. Finally, the best part of herbal system is it can be applied on every age and gender.

Aloe, Tulsi, Neem, Turmeric and Ginger are of very common and easy use in the countries like Bangladesh, India, Pakistan and sometimes in China. These plants are being used years after years in these countries to cure many primary diseases. This is also can be said that this plants have been became a part of life of the people of these regions.

Herbal plants are being used to treat many sexual, bronchial and abdominal diseases. These plants are not only being used to cure diseases but also being used to cook, decorate, make refreshing drinks such as teas, make cosmetic products and so on.

As it has been mentioned earlier that the use of medicinal plants is increasing day by day at a great range but necessary steps has to be taken to develop and enrich the documents about the study of recent and developed findings before it is too late. Only three volumes of WHO monographs has been established on specific herbs from 1999 till present.

1.1.1 Significance of a few herbs with their therapeutic qualities

- Herbs, for example, dark pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are utilized to mend wounds, bruises and bubbles.

- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some critical restorative herbs and can be planted in kitchen cultivate. These herbs are anything but difficult to develop, look great, taste and smell astonishing and a large number of them are magnets for honey bees and butterflies.

- Many herbs are utilized as blood purifiers to adjust or change a long-standing condition by wiping out the metabolic poisons. These are otherwise called 'blood chemicals'. Certain herbs enhance the invulnerability of the individual, along these lines decreasing conditions, for example, fever.

- Some herbs are likewise having anti-toxin properties. Turmeric is valuable in hindering the development of germs, destructive towards microorganisms and microscopic organisms. Turmeric is broadly utilized as a home solution for mend cut and wounds.

- To lessen fever and the generation of warmth brought on by the condition, certain antipyretic herbs, for example, Chirayta, dark pepper, shoe wood and safflower are prescribed by Indian medication professionals.

- Sandalwood and Cinnamon are extraordinary astringents separated from being sweet-smelling. Sandalwood is particularly utilized as a part of capturing the release of blood, bodily fluid and so on.

- Indian sages were known to have cures from plants which act against toxins from creatures and snake nibbles.

- Herbs like Cardamom and Coriander are famous for their inviting qualities. Other fragrant herbs, for example, peppermint, cloves and turmeric add a charming smell to the nourishment, subsequently expanding the essence of the supper.

- Some herbs like aloe, sandalwood, turmeric are usually utilized as germicide and are high in their therapeutic qualities.

- Ginger and cloves are utilized as a part of few hack syrups. They are known for their expectorant property, which advances the diminishing and launch of bodily fluid from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are additionally expectorants.

- Herbs, for example, Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are useful in advancing great blood remedies against diseases. In this manner, they might also be used to fasten the heart rate.

- Certain therapeutic medicinal plants have disinfectant property, which crushes infection bringing on germs. They likewise hinder the development of pathogenic organisms that cause transferable sicknesses.

- Certain sweet-smelling plants, for example, Aloe, Golden seal, Barberry and Chirayata are utilized as mellow tonics. The intense taste of such plants diminishes poisons in blood. They are useful in obliterating disease too.

- Certain herbs are utilized as stimulants to build the action of a framework or an organ, for instance herbs like Cayenne (Lal Mirch), Myrrh, Camphor and Guggul.

- A wide assortment of herbs including Giloe, Golden seal, Aloe and Barberry are utilized as tonics. They can likewise be nutritive and restore a solid and additionally infected person.

- Honey, turmeric, marshmallow can adequately treat a new cut and wound. They are named as vulnerary herbs.

As our way of life is presently getting techno-insightful, we are moving far from nature. While we can't escape from nature since we are a piece of nature. As herbs are regular items they are free from symptoms, they are similarly protected, eco-accommodating and locally accessible.

Customarily there are parts of herbs utilized for the sicknesses identified with various seasons. There is a need to elevate them to spare the human lives.

Today, these home grown items are the image of wellbeing as opposed to the engineered drugs, which are viewed as dangerous to individual and environment. In spite of the fact that herbs had been estimated for their restorative, enhancing and fragrant qualities for a considerable length of time, the manufactured results of the advanced age outperformed their significance, for some time. Be that as it may, the visually impaired reliance on synthetics is over and individuals are coming back to the naturals with any desire for wellbeing and security. It's a great opportunity to advance them all around (Zahid, 2016).

The plant which has been chosen for the research named *Kaempferia galangal* for further study and qualitify the herbs with potential antioxidants. The extract of the whole plant without root of *K. galangal* has been used to continue this study and it carries:

- Extraction of dried powder of the whole plant of *Kaempferia galangal* through Extraction method using methanol including fractionation of methanol extract separation of methanol and acetone fraction.
- Total phenolic content determination of methanol and acetone extract by Folin-Ciocalteu reagent.
- Determining of antioxidant activity through DPPH radical scavenging assay of all the fractional extract.
- Observation of cytotoxic effect of methanol and acetone extract through brine shrimp lethality test.

1.2 Family of the present plant: Zingiberaceae

Approximately fifty genera and around sixteen hundred in between the known species of flowering plants are contained in the family of Zingiberaceae or ginger. The species are basically aromatic in natures which are distributed throughout the tropical areas of Africa, America and Asia.

The species of this family have many important roles in the sector of ornaments, medicine and spice. the shell gingers (Alpinia), Siam or summer tulip, Globba, ginger lily (Hedychium), *Kaempferia* are the few examples of ornamental genera and ginger (Zingiber), galangal or Thai ginger (Alpinia and others) are the few examples of spice.

The classification of the Plantae Kingdom following the Zingiberaceae Family (United States Department of Agricultre) is shown below:

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Liliopsida – Monocotyledons
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae – Ginger family
Genus	Aframomum Schum. – aframomum P
	Amomum Roxb. – cardamom P
	Boesenbergia Kuntze – boesenbergia P
	Elettaria Maton – elettaria P
	Etlingera Giseke – waxflower P
	Hedychium J. Koenig – garland-lily P
	Hitchenia Wall. – hitchenia P
	Kaempferia L. – kaempferia P

The plants contained in the family are basically herbaceous in nature and also contains leaves which are distichous. The distichous leaves also include basal sheaths which form a pseudostem by overlapping. The plants can be supportive to them or can be epiphytic, bisexual flowers, more often than not emphatically zygomorphic and subtended by obvious, spirally organized bracts. The perianth is made out of two whorls, a combined tubular calyx, and a tubular corolla with one projection bigger than the other two. Blossoms regularly have two of their stamenoids (sterile stamens) intertwined to frame a petaloid lip, and have just a single ripe stamen. The ovary is second rate and bested by two nectaries, the shame is channel molded.

Few genera such as Alpinia and Hedychium are used in perfume industry as they produce oils which are essential.

The Zingiberaceae are distributed tropically in the African, American and the Asian regions. In South Africa the diversification is more than in any other regions (Sass et al., 2016).

1.3 Characters of Zingiberaceae Family

The plants of the family Zingiberaceae are rich in aromatic oils and are also lingual, largely differentiated between corolla and calyx from perianth. The staminodium is petaloid and large normally and is also single.

A. Vegetative characters

The habits of zingiberaceae family are perennial and herbs are aromatic in nature, adventitious and rarely fibrous roots. Rhizomes are tuberous or can be horizontal too, mostly not aerial, bracted in nature, normally leafy, can be short or can be elongated too. The stem of the aerial portion can be very small or might not be present at the Curcuma period. Leaves can be radical or can be cauline and exstipulated. Again the leaves can be petiolated or sessile. The bases of the leaves are sheathing sometimes (Cuecuma). The leaves are ranked in two and Costus are spiral in nature. The apexes of the leaves are obtuse or can be acute and the unicostate is parallel.

B. Floral characters

Inflorescence might contain spike which is terminal or panicle or it can also be racemose. The flowers are normally complete and irregular; bracteates and zygomorphic; hermaphrodites; pedicellate or may be sessile; cyclic; distinct kind of calyx and corolla. The flowers normally are of big size and the colors are usually bright and flowers are also aromatic in nature. The sepals of the calyx are 3 and are gamosepalous. The calyxes are tubular in shape and can be like spathe. They are superior and green in color and generally are of 3 toothed. The tube of the calyx is very small in Globba and the anterior of the sepal is odd normally.

The corolla contains 3 petals normally and free or loosely united. The shape of the corolla may be of cone or tubular. They contain three-lobes usually and sizes of the lobes are not equal. They might be linear or might be of broad. The tube of the corolla is large in Roscoea. Corolla and calyx tubes might be of same size or corolla tube might be bigger than the tube of calyx.

Androecium contains six stamens which are distributed in 2 whorls. The posterior stamens are a portion of inner whorls are at the sametime fertile, antipetalous and epipetalous. The two left stamens are sterile which are located in inner whorl and basically petaloid and fused by one another to make a lip of petaloid or labellum and they might be of two to three lobe containing.

Gynoecium contains three capillary and they are syncarpous and inferior in nature. The fruits are basically capsules which are loculicidal and opened by the help of three valves. The seeds are usually endospermic and the pollination pattern is entomophilous.

1.4 Distribution of Zingiberaceae

49 genera and almost over 1300 species are contained in the family Zingiberaceae within which more than half has been found in India. Records showed that around seventeen genera and one hundred and fifteen species are from India. The particular family is largely distributed in the eastern hemisphere within which maximum of them are found in Indo-Malayan area.

1.5 Necessity of Zingiberaceae economically

The members of Zingiberaceae are not only being used as perfume, medicine or spice they are also being used in foods and cosmetics.

1. **Food**
    - H. Adarak is a kind of commercial ginger is basically used as spice. They are also used in perfumery.
    - H. Haldi which is known a turmeric are being used years after year as spice and this turmeric gathered from the roots of *C. longa* and *C. domestica*.
    - The locally used Elayachi or cordamon are used as spice and condiment and are obtained from Elettaria cardamomum.

2. **Medicinal**

    - The curcuma longa in the powder is used as Ayurvedic medicines and also externally applied in wounds and sprains.

    - *Zingiberaceae officinale* is also used as carminative.

    - In producing tonic products Zeodary is being used.

3. **Perfume**

    - From Hedychium Spicatum few scented powders such as Abir, Zingiber officinal ear produced.

4. **Ornamental**

The plants of this family such as Costus, Alpinia, Globba, Brachychilum, Hedychiumcoronatum, Roscoea are used to decorate or beautify gardens and houses.

1.6 Most Commonly used plants of Zingiberaceae family

1. *Alpinia galanga* – scented flowers are one of the characters of these plants and are cultivated in the gardens.

2. *Curcuma amada* – also known as Mango ginger because the rhizomes contains the sweet smell like mangos.

3. *Costus speciosus* – they are cultivated shady places where other plants are difficult to grow.

4. *Globba bulbiflora* – they are seen in the marshy areas and the places like river-banks (Yashasvi, B.).

1.7 Investigational Plant: *Kaempferia galangal*

Kaempferia galangal, generally known as kencur, sweet-smelling ginger, sand ginger, cutcherry, or revival lily, is a monocotyledonous plant in the ginger family, and one of four plants called galangal. It is discovered fundamentally in open ranges in Indonesia, southern China, Taiwan, Cambodia, and India, but on the other hand is generally developed all through Southeast Asia.

Table 1.1 Taxonomy and Nomenclature (National Plant Data Center, 1996)

Kingdom	Plantae
Taxonomic rank	Species
Common name(s)	Galanga (English)

Table 1.2 Taxonomic Hierarchies (Flora of North America Expertise Network, 2010)

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Lilianae – monocots, monocotyledons, monocotyledons
Order	Zingiberales
Family	Zingiberaceae – Ginger Family
Genus	Kaempferia L. – kaempferia
Species	*Kaempferia galanga L. – galangal*

1.7.1 Vernacular Name

- **Bangla** **:** Chandramulika
- **Malaysia** **:** Cekur, cengku r(peninsular), cekur Jawa
- **English** **:** East-Indian galangal
- **Philippines** **:** Gisol, dusol, disok
- **Indonesia** **:** Cekur, Kencur, bataka
- **Thailand** **:** Horn proh, waan teen din, waan horn

1.7.2 Geographical Distribution

These herbs are widely spread in India and also might be native to this country. These herbs are cultivated in the regions such as Southeast Asia, northern Australia, southern China, and Malaysia.

1.7.3 Description

These herbs are small in size. The leaves contain sheaths of 1.5-5 cm long. The blades are horizontally lying flat along with the soil. They are basically elliptical and the circular outline is a bit flat with the smooth upper surface and cobweb-hairy lower surface.

Inflorescences are sessile which are coming out from the middle of the leaves with 4-12(-15)-flowered and long sepal (2-3 cm). The white petals contain 2.5-5cm and 1.5-3 cm long tubes and lobes respectively. The lips are egg-shaped and divided into two or more with white or purple color while the base contains violet or purple color. The lobe size of lateral is almost of 2-2.5×1.5-2 cm. The anthers of stamens are not perfect and the shapes are oblong-egg or oblong-lance along with white color and 1.5-3cm long length. The stamens are 10-13mm in length and fertile.

Figure1.1 *Kaempferia galangal* plant

This plant are normally grown and cultivated in old gardens and villages of the regions. Along with the other parts of the plan the root is one of the most useful parts of the plant.

These plants are called in different name in different regions in some language it refers to sand as in German and Chinese they are called Sandingwer and “Saajiang” respectively. Systemically names are given to honor Engelbert Kaempfer who was a German botanist.

1.7.4 Cultivation

K. galangal grows well in shaded or cool places. Forests and different type of mud or soils within altitude of 1000m are the best places for this plant to grow best (Plant Resources of South-East Asia).

1.7.5 Traditional use and effect

In the regions such as New Guinea the plant *K. galangal* is being known as entheogen and aphrodisiac. This plant is used in the religious rituals in that region (Voogelbreinder 2009, 207). This plant contains aromatic roots which are very rich in nature and because of this they are being used as spices. They have a higher value for medicinal purpose too. They were used to make poison for arrow in Malaysia. The roots are used to increase the flavors of the cooked food and also as medicine in the regions like Thailand (Ratsch 1998, 563).

Though the chemistry is not that much known yet but the plants contains essential oils highly and this may exert hallucinogenic symptoms (Hofmann et al. 1992, 46). The roots of this plant is a very known hallucinogenic agent with low or no side-effect and being used for this purpose in between and by the people of Papua New Guinea and Mount Hagen (Ratsch 1998, 563-564).

1.7.6 Medicinal uses

- *K. galangal* is not only being used as an expectorant but also as a carminative under medicine in Asian regions.
- A specialized tea is made by the leaves of the plants and used to treat diseases like sore throat, infection of eye, swelling and rheumatism in India.
- To treat headache the mixture of powdered root and whiskey is used in Thailand.
- It is very famous between the Arabic people to treat fever and inflammation immediately as they exert tonic and stimulant activity.

- It is also used to treat chest and abdominal cold, toothache, vomiting, intestinal parasite and diarrhea (Voogelbreinder 2009, 207).

1.8 Uses in Bangladesh

K. galangal (Chandramulika in Bengali) having a place with the group of Zingiberaceae is a rhizomatous and little herb. The rhizomes of this plant are generally utilized as a part of East Asia for an extensive variety of restorative applications. The most widely recognized signs incorporate ailment, asthma, cerebral pains, hack, toothaches and use as a poultice for the application on wounds and wound (Perry and Metzger, 1980). The fragrant oil is utilized as sauce and as a people prescription. In Bangladesh, rhizomes juices of *K. galangal* are utilized as a solution for toothache or a wash for dandruff or scabs on the head. It is utilized as stimulant, stomachic and carminative and remotely used to treat stomach torment, swelling and ailment (Sirirugsa, 1997). The review uncovered that concentrates of *K. galangal* has mitigating and pain relieving, nematicidal, mosquito repellent and larvicidal, vasorelaxant, calming, antineoplastic, antimicrobial , hostile to oxidant properties. It additionally has anti-diarrhoeal and cytotoxic properties. A few mixes has been secluded from dicloromethane, hexane and methanol concentrates of rhizome *K. galanga* i.e. ethyl-cinnamate and ethyl–p—methoxycinnamate (Umar et al., 2011). Disconnected ethyl-p-methoxycinnamate from *K. galanga* extricates in charge of different pharmacological activities including, nematicidal, mosquito repellent, against neoplastic and hostile to microbial impacts while ethyl cinnamate, a fundamental constituent of this plant, in charge of its vasorelaxant impacts (Umar et al., 2012). The real synthetic constituents of the unpredictable oil from dried rhizome of *K. galanga* were ethyl-p-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), individually. Different constituents of the rhizome incorporate cineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, p-methoxycinnamic corrosive and ethyl cinnamate (Ridtitid, 2008). To date, unique concentrates of *K. galangal* have not been systemically considered.

1.9 Literature Review

Almost over sixty species of *Kaempferia galangal* are distributed throughout Africa, South-East Asia and India. Between the species, *K. galangal*, *scaposa Benth.*and *Hook., K. rotunda L.* and *K.* are being used as spices over South India. *K. elegans W.* which is popularly known as 'peacock ginger' in Malaysia is being used as an ornamental spice. *K. galangal* is being used as spice, cosmetic components, medicines and condiments. Few studies showed that at past the plant has also been used to produce abusive drugs in Kerala. Normally leaves, Rhizomes and root stocks of this plant are being used for various purposes. Here the following sectors on this plant have been highlighted.

- **Status of spices, origin and distribution:** In the ginger family *K. galangal* is a highly priced plant for medicinal and aromatic purpose and also highly distributed in Tropical Asia. Biogeography and the evolution of the plant have been studied by Wood (1991). Though the plant is mostly growing in China and Asia, it is believed that the origin of the plant is from East Asia or Burma. Kerala, Tamil Nadu, West Bengal and Karnataka are places of India where this plant is being cultivated in a large scale.

- **Agronomy:** Shades are needed to grow *K. galangal*. The plant requires few things to grow up to 1500 m and the factors are warm and humid weather, 1500-2500mm yearly rain fall and rich soil. To cultivate the plants the rhizomes are collected and stored in a cold and dry place (Ravindran, 2005). Morphological characters of this plant are not that much affected by the kind of seedlings but the mother rhizomes are more prioritized than the finger rhizomes (Razagopalan, 1985). The growth of *K. galangal* is higher and the yields of rhizome are also high when it is cultivated as intercrop (Maheswarappa et. al, 2001). The growing and yield rate of *K. galangal* is comparatively higher under the plantation of coconut rather than an open place (Gunathilake, 2000). Mulching with the leaves of Gliricidia machuta, Azadirachta indica and Chromolaena odorata provides the best fresh weights. The most effective rhizome yields for the infection of nematode were obtained by the mulches with Azadirachta indica (Nisha, 2002). Few other studies showed that the yields of rhizome at a greater rate is linked with the high content of P, Ca and K and it introduces the rhizomes of rich essential oils containing Zn, S, Mn and Mg

(Raghavan, 1943). Almost about 4-6 tons per hectare of refreshed rhizomes of *K. galangal* can be obtained from a correctly managed and careful plantation (Ravindran, 2005). Normally these plants are not affected by pastes and insects but bacterial wilt has been caused by *Pseudomonas solanacearum* (Dake, 1995).

- **Cytology:** The determined compliment of somatic chromosome normally is 2n=22 (Sharma, 1959). The numbers of long, medium and short chromosomes are six, four and one pairs respectively. The reported general number of this specific genus is six (Raghavan, 1943). The species of Asia and Africa contains 2n=22 and 2n=28 or 42 with the basic number x=11 and x=14 respectively (Spearing 1964). As the tip cells of the root expressed 54 chromosomes few authors uses the word aneuploidy pentaploid for *K.galangal* (Ramachadran,1969)

- **Economic importance:** The dried rhizomes of *K.galangal* are highly demandable and that is more than 100 tons (Lalitha, 1998). The current price value of the dried root is of Rs.300/ per one kilogram. It is also have a very good demand worldwide and that's why it has a good potential in export too (Thomas, 1998). The demand of the essential oils extracted from this plant is very high between the local people and pharmaceutical industries and the current price range is US$600-700 per one kilogram in international market (Chithra, 2005). The plant used to be cultivated locally under restriction in Kerala but the cultivation has been approved to the commercial level because of perfumery, flavoring and medicinal reorganizations.

- **Medicinal uses:** By using *K. galangal* Ayurvedic medicines are being produced and the number is more than 59. The components of this plant are also introduced in Ayurvedic drugs, cosmetics, spices and perfume (Rahman, 2004). This plant is used to raise the energy level to reduce exhausted feeling, treat diarrhea and migraine (Spearing, 1964). The root stocks and rhizomes are bitter, febrifuge, aromatic, thermogenic, depurative, vulnerary, acrid, diuretic, digestive, carminative, stimulant, anti-helmentic and expectorant. They are very successful to treat dyspepsia, heasthma, splenopathy, hemorrahoids, leprosy, bronchitis, tumor, skin disease, ulcers, nasal obstruction, rheumatism, fever, cough and helminthiasis (Kirtikar, 1996).

- **Ethnobotany:** In the East Asian region the rhizome of this plant is used at a great range because of its medicinal property (Sadimann, 1992). The rhizomes of the plant are being used by the medical practitioners who are practicing indigenously to treat psoriasis, tumor, infections occurred by bacteria, rheumatism and also used externally to reduce the abdominal pain of women (Hirschhorn, 1983). The flowers and leaves of the plant are highly rich in flavanoids (Ghani, 2000). As the leaves of this plant provides antioxidant, anti-inflammatory and anti-nociceptive property they are used to treat headaches and ulcer of mouth. To reduce coughs freshly collected leaves are chewed and after childbirth the leaf ashes are rubbed on the breasts which are swollen (Sulaiman, 2008). The drink made by the infusion of leaves might be beneficiary to the pregnant women (Rahman, 2004). To make a famous dish *K. galangal* is used locally (Larsen, 1999).

- **Phytochemistry:** The study of chemistry of *K. galanga* has been done widely. 2.5-45 of volatile oil is contained in its rhizome (Ravindran, 2005) along with few alkaloids, minerals, starch, fatty matters, amino acids and proteins (Wong, 1992). The root contains more volatile oil compared to rhizomes (Arambewala, 2000). There are almost 54 compounds are present in the essential oil and the major components are 16.5% of ethyl–trans p–methoxycinnamate, 3.3% of g– careen, 9% of pentadecane, 2.7% of borneole and 5.7% of 1,8–cineole. It also contains components such as camphene, p-methoxycinnamic acid, kaempferol, cinnamaldehyde, ethyl cinnamate and kaempferide (The Wealth of India, 1959), (Sudibyo, 2000), (Twetrakul, 2005). It also contains 16.4% of terpene oil (Wong, 1992).

- **Pharmacology:** The rhizomes of *K. galangal* after drying is being used to stimulate Central Nervous System and as a cardiotonic in Thailand (Mokkhasmit, 1971). The extract is being used to inhibit the effect of monoamine-oxidase (Noro, 1983). Highly cytotoxic ethyl p-methoxytranscinnamate is contained in the methanol extract of the rhizome of the plant (Kosugen, 1985). From the extract of the rhizomes anticancer and larvicidal components are found (kiuchi, 1988). The extract of the plant's rhizome are useful to stop the viral (Epstein-Barr) activity. The rhizome's extract was also effective to kill the larvae of *Culex quinquefasciatus* mosquito and also to damage the adult mosquito *Aede saegypti* and two of them are very serious vectors towards diseases (Choochote,

1991), (Xue, 2002). For rheumatism and fostering tumors the roasted and hot rhizomes are used (Chitra, 2005). The extract of the plants is used to reduce the irritation caused be the caterpillar stinging (Chitra, 2005). Further researches are being done to find out the useful properties as insect repellent and first degree research showed that these are non-irritant to the rat skin (Kanjanapothi, 2004). The potent chemicals to the insects are found in the rhizomes of the plant (Ahn, 2008). The healing property has also been found in wounds (shanbhang, 2006). The alcoholic extract of the crude rhizomes showed cytotoxic properties against cancer and normal cell cultures (Jagadish, 2010). The antioxidant property and HPLC analysis of hudro-ethanolic extract of crude leaves has also been reported (Kaushita, 2015).

1.10 Rationale of the present preview

K. galangal is utilized as an herb in cooking in Indonesia, where it is called kencur, and particularly in Javanese and Balinese foods. Beraskencur, which joins dried *K. galangal* powder with rice flour, is an especially well known jamu home grown drink. Its leaves are likewise utilized as a part of the Malay rice dish, nasiulam.

Not at all like the comparable *Boesenbergia rotunda* (Thai krachai), *K. galangal* is not normally utilized as a part of Thai cooking, but rather can be purchased as a dried rhizome or in powder shape at home grown pharmaceutical slows down. It is referred to in Thai as prohhorm (เปราะหอม) or waanhorm (ว่านหอม), and in Khmer as prâh. This being used in cooking and medicine by Chinese people (Van Wyk, Ben-Erik, 2005).

As the root of the plant has gone under so many scientific study but the leaves of the plant never brought under any study and that is why the plant has been chosen for the research for further study and qualitify the herbs with potential antioxidants.

1.11 Target and objectives of the present preview

- The establishment of the leaves of plant *K. galangal* as medicinal plant is the main target of the following study.

- The objectives of the study are:
- ✓ Total Phenol Content Quantification,
- ✓ *in-vitro* Antioxidant Activity investigation,
- ✓ Cytotoxic Effect of the Methanol and Acetone Extract of *K. galangal* Leaves

1.12 Necessity of potential antioxidant of natural source

1.12.1 Understanding antioxidants

Normal cancer prevention agents are found in loads of plants and various sustenance and flavors we eat each day. They help battle oxidation, an average response that occurs in the body every day. Exactly when there are unsettling influences in the common oxidation prepare, incredibly shaky and perhaps hurting molecules called free radicals (additionally clarified beneath) are shaped. Oxygen triggers the game plan of these devastatingly dangerous little chemicals and if left uncontrolled, they can hurt cells in the body. It's much similar to the response that makes rust on a bicycle or turns the surface of a cut apple dull. Cell reinforcements work by giving an electron to an atom that has been bargained by oxidation, bringing it once more into a condition of appropriate capacity. Being spent in this procedure, hostile to oxidant particles are recharged by taking an electron from option cell reinforcement or is adjusted into building matter, for example, collagen utilized as a part of repairing tissue matter (Berkeleywellness, 2015). Despite what might be expected, combined adaptations of hostile to oxidants cannot be utilized as a part of this way after they lose their electrons. In the wake of being "spent" they have a tendency to end up distinctly risky side effects that produce additionally weight on the oxidative heap of the substance. Cancer prevention agent lack is connected with significant illnesses, for example, Alzheimer's, growth, Parkinson's and that's only the tip of the iceberg (Y. Feng and X. Wang, 2012).

1.12.2 Antioxidant as protection

An exceptionally advanced and perplexing barrier instrument utilizing cancer prevention agents has been made in people to battle against receptive oxygen species and ensures our bodies. It utilizes a determination of segments, both endogenous and exogenous, that work intelligently to

balance free radicals. The absolute most broadly inquired about dietary cancer prevention agents are vitamins E and C. Vitamin C can balance ROS in its fluid stage before lipid per oxidation and is thought to be an extremely powerful, if not most essential water-dissolvable cell reinforcement. Vitamin E is the most effective chain-breaking cancer prevention agent inside the cell membrane. Vitamin C is known to help repeat vitamin E. Products of the soil contain a major supply of vitamin C while entire grains and top notch vegetable oils are real wellsprings of vitamin E (Wang and Quinn, 1999).

1.12.3 Endogenous cell reinforcements

Notwithstanding dietary cancer prevention agents, the body relies on upon a couple of endogenous hindrance frameworks to secure itself against free radical-influenced cell harm. The cancer prevention agent proteins – catalase, glutathione peroxidase and superoxide dismutase (SOD) – metabolize hazardous intermediates and require micronutrient cofactors, for instance, selenium, iron and zinc for perfect synergist action. It has been recommended that a lacking dietary affirmation of these take after minerals may thwart the execution of this protection system (Fontenot, 2011).

1.12.4 Free radicals

A free radical is characterized to be any molecule with at least one unpaired electron in the external shell and equipped for existing all alone. Developments of free radicals happen effectively when a covalent bond separates and one electron is extra with the recently made particles. Their profoundly receptive qualities originate from the free electron in the external shell. The way that they are exceptionally responsive implies that they can respond with most particles in its region. This incorporates proteins, lipids, sugars and DNA. Consequently, free radicals bond with the closest accessible atom, "taking" its electron. At the point when the "assaulted" particle drops an electron and itself turns into a free radical, making a chain response. The procedure can course to unsafe statures which may bring about the disturbance of living cells (Hasslberger, 2007).

Since we to some degree comprehend the emergency made by free radicals in our framework we have to take a gander at techniques for countering them. As expressed, orchestrated cell reinforcements have practically no impact in countering free radicals. Truth be told, when they are spent, they themselves turn into an unsafe specialist in our bodies, making a most risky therapeutic agent named paradox. Subsequently, we turn our interests to normal means, backpedaling to the "roots" of solution as we probably am aware it today.

These days, it has turned into a well-known misinterpretation that home grown drug is medieval, a primitive method for treatment. What we neglect to acknowledge is that through a huge number of years of development our bodies have turned into the ideal surviving instruments nature brings to the table. Its method for survival originates from nature and over a great many years have turned out to be acclimated to its items. All that we should be sound can be found in nature. In this way, plants and common sustenance have been utilized as a part of medicinal purposes before history was accounted.

1.13 Cytotoxicity study

Toxicology takes its underlying foundations in the utilization of death, slaughter, wrongdoing and suicides. To a great extent famous in pre-scriptural Greece, a notable illustration is that Nicandar of Colophon (185-135 BC) exploring different avenues regarding harms on lawbreakers of the Bynthian Kingdom. The broad utilization of toxin in this way esteemed in essential to devise medicines and

Maimmonides expressed “Poisons and their antidotes” in the twelfth century (John Timbrell, Introduction to Toxicology, Third release, p3-4).

In any case, it is not till late circumstances that toxicology made significant walks in logical interests, all the more imperatively cytotoxicity - how much an operator has particular dangerous activity on specific cells.

A standout amongst the most broadly utilized methods of testing cytotoxicity is the saline solution shrimp lethality bioassay. It picked up acknowledgment because of its quick and extensive nature for the bioactive compound of characteristic and engineered root. It takes into

consideration the bioactivity testing of characteristic item extricates, portions and also the unadulterated mixes. This test helps in deciding pharmacological exercises like antimicrobial, hostile to viral, pesticidal and against tumor exercises of bioactive mixes of common and engineered source (Pisutthanan, 2004).

Chapter Two:

Methodology

2.1 Chemical works

The chemical works which has been done to the leaves of *K. galangal* is being shown below shortly:

1. Careful collection, identification and storage of the investigational plant *K. galangal*.
2. Proper preparation of the total plant sample.
3. Methanol and acetone extraction of the whole plant.
4. Evaporation of solvents to get the final crude methanol extract (CME) and crude Acetone extract (CAE).
5. Relative chemical tests to determine the total phenolic content, antioxidant activity and cytotoxic effect of the crude methanol extract (CME) and crude acetone extract (CAE).

2.2 Investigational plant *K. Galangal* collection, identification and preparation

The whole plant of *K. galangal* was collected from Savar, Dhaka. Bangladesh National Herbarium has identified the plant as *K. galangal*. The leaves of the plant *K. galangal* were washed clearly with clean water. After washing, the plant was dried for some days in sunlight. Then the plant was dried in the oven at a very low temperature (less than 40^0C) for around 24 hours. The plant portions were converted into powder with the help of powerful grinding machine in Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka.

2.3 Extraction of the plant powders

By the help of cold extraction process the powders of investigational plant material was extracted. The powdered materials were taken in two different convenient bottles and were soaked in 500ml of methanol and acetone separately. The bottles of contents were kept in a dark and cold place for fifteen days and regular stirring and shaking was being done. The mixtures were filtered by using cotton and Whitman no.1 filter paper respectively. Finally, the crude methanol extracts (CME) and crude Acetone extract (CAE) were gained by concentrating the filtrate with the help of a rotary evaporator under low pressure and in a convenient temperature.

2.4 Chemical tests of CME and CAE

2.4.1 Total Phenolic Content determination of CME and CAE

A various group of phenolic compound a great chemical structure to hunt free radicals (Ahmed et al., 2014).

- **Principle:**

The phenolic contents of the plant extracts are usually determined by the help of Folin–Ciocalteu Reagent (FCR). The chemical components of FCR are not entirely known yet but it is imagined that it might contain hetero polyphospho tungstates – molybdates. Usually blue species shows few reversible electron reduction reactions. Molybdenum is easily reduced in complex reactions (Ahmed et al., 2014).

- **Reagents and chemicals:**
  - ✓ Folin-ciocalteu reagent
  - ✓ Methanol
  - ✓ Sodium carbonate
  - ✓ Gallic acid
  - ✓ Distilled water

- **Experimental procedure:**

Folin-ciocalteu reagent was taken of 2.5 ml in a test tube. Plant extract of 0.5 ml was dissolved in it. The mixture was diluted for 10 times and was collected in separate test tubes. Sodium Carbonate of 2.5ml was added to all of them and the mixtures were incubated maintaining 24^0C temperature for about 20 min. all the reagents were prepared instantly. The absorbance of the spectrometer was set at 760nm against the blank containing all the compounds without plant extract.

The following equation is used to determine the total phenolic content of the extracts:

$C= (c \times V)/m$

Where,

C= the total content of phenolic compounds, mg/g plant extract in GAE

c = concentration of Gallic acid obtained from the curve (mg/ml)

V= the volume of the sample solution (ml)

m = weight of the sample (g)

The experiments were performed for three times and the average results were documented.

2.4.2 Antioxidant Assays

2.4.2.1 DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

DPPH is the highly acceptable chemical compounds which are being used to restore natural and artificial origin's extracts free radicals (Marinova, 2011). Antioxidant activities of various compounds are indicated based on the hydrogen donating capability by the help of DPPH method under a slight modification.

- **Principle:**

The DPPH reduction of the methanol solution is the base of this method in the presence of an antioxidant which can donate hydrogen because of the formation of non-radical form of DPPH-H. An instant prepared solution of DPPH appears in deep violet color. The color converts into yellow when transformation is occurred and the transformation is measured by the help of spectrophotometer. That is why the antioxidant compounds will neutralize the free radicals of the DPPH by the result of conversion of DPPH into a colorless transparent product (2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) and will reduce the absorbance.

The potency of antioxidant activity is inversely proportional to the rate of decrease in wavelength. For the purpose of control positively ASA (ascorbic acid) and BHT (butyl-1-hydroxy toluene) in this experiment (Marinova, 2011)

- **Procedure:**

The methanol and acetone solutions of 2 ml sample extract were taken and in different concentrations standards were prepared. 3ml of instantly prepared DPPH solution were added to each and every test tube. To continue the reactions test tubes were placed in a dark and cool place for half an hour. Then the wavelength of the UV spectrophotometer were set at 517 nm compared to a standard solution containing all reagents without the test sample. Lastly, the absorbance of all the concentration were measured and noted down.

$\% I = \{(A_B - A_S)/A_B\} \times 100$

Where,

A_B = blank/control absorbance, and

A_S = extract/standard absorbance

IC_{50} values are calculated by drawing the graph of %I of inhibition verses concentration (µg/ml).

2.4.3 Brine Shrimp Lethality Bioassay

- **Principle:**

For Cytotoxicity determination Brines shrimp bioassay is one of the worldwide accepted and known methods. In the artificial sea water the eggs of shrimps are hatched to gather the nauplii. Test tubes were taken and dimethyl sulphoxide (DMSO) were taken into them of the required amount to get the required the concentration. After the preparation of hatching ten of the nauplii were counted and taken to the test tubes containing 5ml of artificial sea water and various concentrated sample by using micropipette and the tubes were labeled. The tubes were kept in a cold and dark place for around 24 hours to investigate the survival capability of the shrimps in the test samples. After this time period the numbers of dead naupliis were counted visually and were taken to further calculation (Olowa & Nuneza, 2013).

- **Materials:**

- ✓ Brine shrimp eggs (*Artemiasalina leach*)
- ✓ Sea salt (NaCl)
- ✓ Small tank with perforated dividing dam to hatch the shrimp
- ✓ Test samples of experimental plants
- ✓ Micropipette and pipette
- ✓ Micropipette
- ✓ Lamp to attract shrimps
- ✓ Magnifying glass
- ✓ Test tubes

Table 2.1 Test samples of experimental plant

Plant Part	Sample Code	Test Sample	Calculated Amount (mg)
Leaves of plant *K. galangal*	ME	Methanolic extract partitionate	4.0
	AE	Acetone extract partitionate	4.0

- **Procedure:**

1. **Preparation of seawater**

38g of sodium chloride or sea salt was measured and dissolved in distilled water of about 1 Liter and then filtration was done to gain the clear solution.

2. **Hatching of brine shrimps**

The *Artemiasalina leach* was collected from a pet shop brine shrimp eggs. The tank containing artificial sea water was covered properly and the eggs were added to the tank with continuous oxygen supply. The eggs were hatched for one day and were kept under a lamp. After a day, the 10 nauplius were counted and added to each of the test tubes of different concentrations by using Pasteur pipette.

3. Test sample preparation of Experimental plant

At first to prepare the stock solution both of the samples (Table 6.1) were placed in vials. The test samples were dissolved in pure dimethyl sulphoxide or DMSO of 100 µL. Simulated sea water of 5ml was taken in first vial or test tube and the solution of 50 µL was taken in it. Afterwards 10 shrimp nauplius was counted and added into it and the concentration was 400 µL of the first test tube. After that nine more test samples were prepared containing different concentrations from the first sample and all of the test samples contained DMSO (50 µL) and sample extract (50 µL) in each case. As a result all the test samples contained different concentrations.

5. Control group preparation

To carry out cytotoxicity analysis or study control groups are necessary for the validity of the experimental analysis to make sure that the obtained results are more acceptable by diminishing the effect of other factors. Control groups which are prepared are of two types and they are-

- Positive control and
- Negative control

6. Positive control group preparation

A worldwide accepted cytotoxic agent vincristine sulphate has been used in this study as the positive control group and after that obtained result of the experimental samples were compared with the positive control group. The vincristine sulphate of required amount was dissolved in DMSO to get 20 µg/mL concentrations. Again the process undergone the serial dilution to obtain 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, 0.3125 µg/mL, 0.15625 µg/mL, 0.078125 µg/mL and 0.0390 µg/mL of concentration respectively. Then 10 alive shrimp nauplius were added to each of the samples along with simulated sea water of 5ml.

7. Negative control group preparation

Here three test tubes were prepared which contained simulated sea water (5ml) and DMSO (100 µL) along with 10 alive shrimp nauplius. If the shrimps are died in a remarkable rate then the

experimental process will be considered as an invalid one as the shrimps were dead for some other causes rather than compound's cytotoxicity.

8. Nauplii counting

The test samples were kept in a suitable place for around 24 hours and the shrimp nauplius were counted with the help of a magnifying glass. After counting the mortality rate was obtained and analyzed statistically through linear regression by using an IBM-PC program. Median lethal concentration or LC_{50} value is an expression which shows the relationship of concentration-mortality of the sample products. The LC_{50} value considers the concentration of the test sample which causes the death of the half shrimp population after a fixed time.

Chapter Three:

Results and Discussion

3.1 Crude solvent extract

Table 3.1 Different extracts obtained after extraction of *K. galangal*

Name of the Partitionates	Weight(gm)
CME (Crude methanol extract)	**8.06**
CAE (Crude Acetone extract)	**6.86**

3.2 Determination of total phenolic content

The Folin-Ciocalteu reagent (FCR) is one of the most acceptable and suitable reagent worldwide and in this experiment of finding out of total phenolic content of crude methanol extract (CME) and crude acetone extract (CAE) of *K. galangal*, FCR has been used. The Gallic acid standard curve has been used here as a standard and the phenolic content of CME and CAE were calculated basing on this curve. The gallic acid standard curve has been shown in the table 5.2 and figure 5.1. The analyses are shown in the ratio of gallic acid extract concentration in mg versus dried extracts in gm. The values are calculated in average and expression is mean ± STD (Ahmed et al., 2014).

Table 3.2 Absorbance of gallic acid at different concentration treating with FCR reagent

Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
	A	b	C	
1	0.078	0.075	0.076	0.076 ± 0.001
2	0.176	0.171	0.181	0.176 ± 0.005
4	0.364	0.368	0.372	0.368 ± 0.004
8	0.722	0.718	0.726	0.722 ± 0.004
16	1.413	1.417	1.423	1.417 ± 0.005
32	2.758	2.752	2.764	2.758 ± 0.006

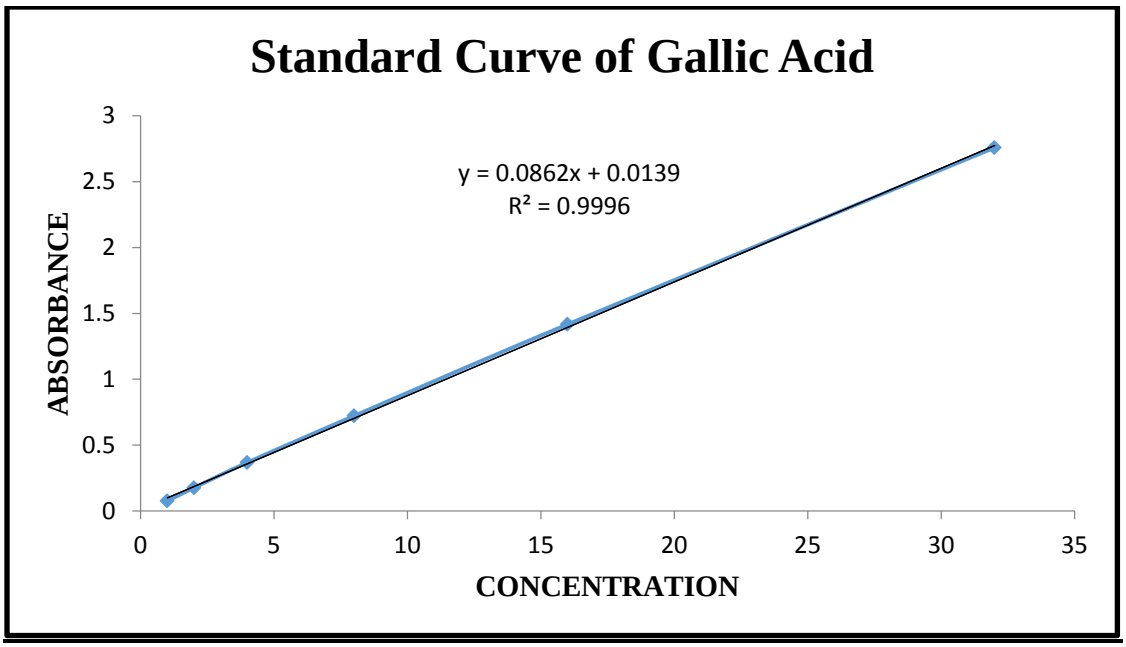

Fig: 3.1 Standard curve of gallic acid for the determination of total phenolic content.

The following table 3.3 and figure 3.2 shows the results, calculation and determination of the crude methanol extract (CMC) and crude acetone extract (CAE).

Table 3.3 Total phenolic content determination of the crude methanol extract (CME) & crude acetone extract (CCE) of *K. galangal.*

Sample	Sample no.	Concentration (µg/ml)	Absorbance	GAE/gm of Dried Sample	GAE/gm of Dried Sample Mean
Crude methanol extract	1	250	0.043	0.349	0.380
	2	250	0.059	0.535	
	3	250	0.035	0.256	
Crude acetone extract	1	250	0.0121	-0.010	-0.019
	2	250	0.0096	-0.040	
	3	250	0.0135	-0.006	

From the table 3.3 it is seen that the total phenolic content of crude methanol extract (CME) and the crude acetone extract (CAE) yields are 0.380 and -0.019 GAE/gm respectively of dried sample and result shows that crude methanol extract (CME) has the greater total phenolic content compared to the crude acetone extract (CAE).

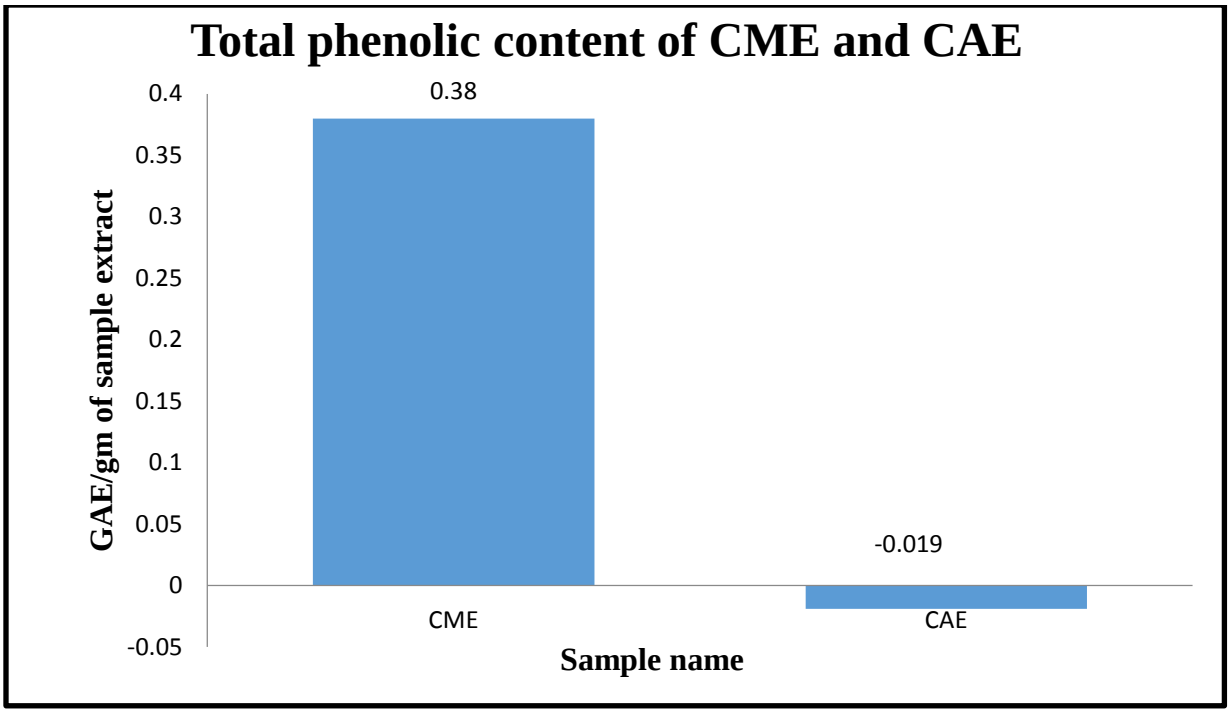

Figure 3.2 Total phenolic content (µg/gm plant extract in gallic acid equivalent) of the crude methanol extract (CME) and crude acetone extract (CAE) of *K. galangal.*

3.3 DPPH Radical Scavenging Activity

At presence of antioxidants, color of sample solution is changed and that expresses the capacity of 1, 1-diphenyl-2-picryl-hydrazyl which is a steady free-radical. The absorbance of DPPH is shown at 517nm as it contains an electron bound very loosely and that is why it exerts deep purple appearances. DPPH converts its colors as an electron is added and that is responsible to show different absorbance which is used to measure the percentage of scavenging ability. The catalyzation is done by the increase in the sample concentration. The well-known DPPH radical scavenging assay has been used here to measure the antioxidant activity of crude methanol extract (CME) and crude acetone extract (CAE).

Here CME and CAE were the main samples who were subjected to this assay. The following table 3.4 and figure 3.3 shows that the lowest and highest IC_{50} value containing samples were crude methanol extract (CME) and crude acetone extract (CAE). So it is seen that the crude methanol extract (CME) has the highest anti-oxidant activity. Here two standards have been used

against the partitionates and they are BHT (butyl-1-hydroxy toluene) and ASA (ascorbic acid).

Table: 3.4 IC_{50} (µg/ml) values of crude methanol extract (CME) and crude acetone extract (CAE) of *K. galangal* and BHT (Standard) for DPPH radical scavenging activity.

SAMPLE CODE	TEST SAMPLE	IC_{50}
BHT	Butyl-1- hydroxyl toluene	28.30
ASA	Ascorbic acid extract	5.90
CME	Crude methanol extract	611.824
CAE	Crude acetone extract	702.791

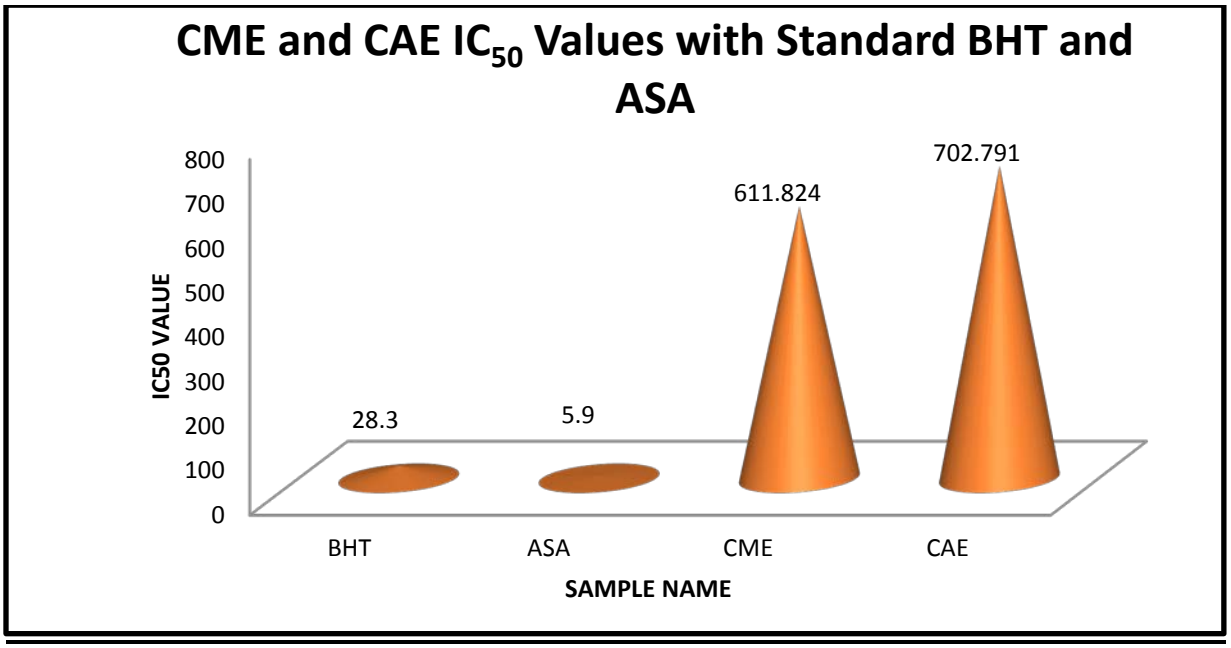

Fig: 3.3 IC_{50} (µg/ml) values of crude methanol extract (CME) and crude acetone extract (CAE) of *K. galangal* and BHT, ASA (Standard) for DPPH radical scavenging activity.

Table 3.5 IC_{50} value of tert-butyl-1-hydroxy toluene (BHT)

Absorbance of the blank	Concentration (µg/ml)	Absorbance of the extract	% of inhibition	IC_{50} value µg/ml
0.324	0.977	0.287	11.42	28.30
	1.953	0.238	26.54	
	3.906	0.225	30.56	
	7.813	0.206	36.42	
	15.625	0.175	45.99	
	31.25	0.159	50.93	
	62.5	0.135	58.33	
	125	0.097	70.06	
	250	0.068	79.01	
	500	0.018	94.44	

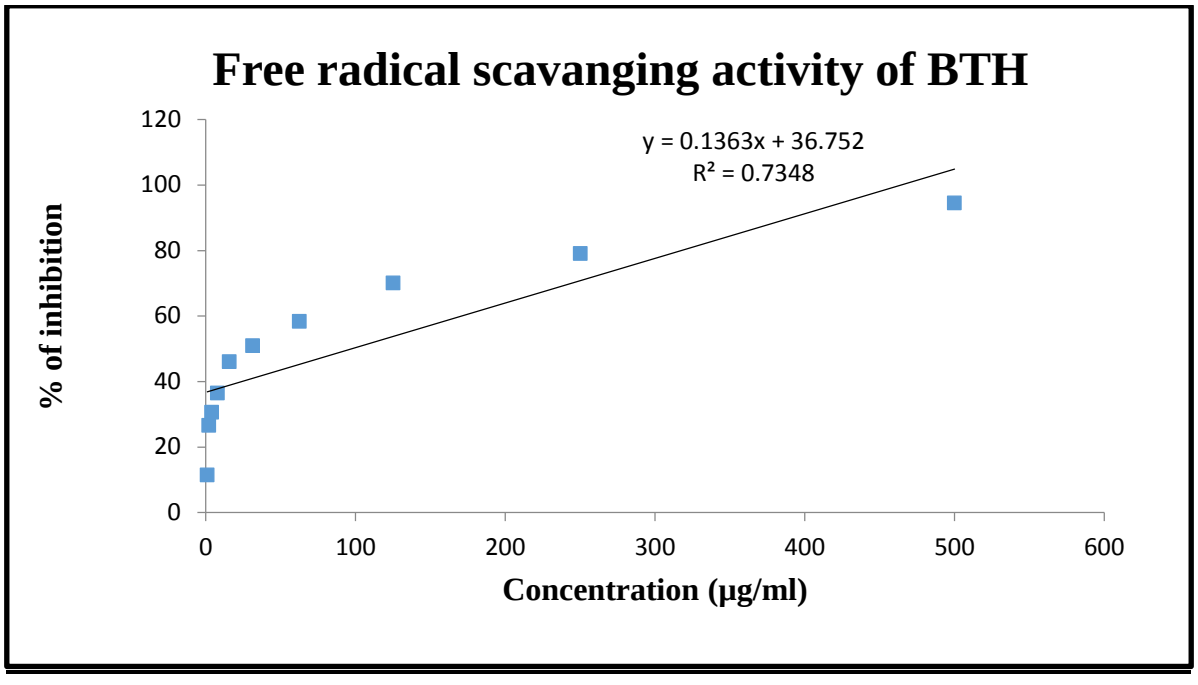

Figure 3.4 IC_{50} value of tert-butyl-1-hydroxy toluene (BHT).

Table 3.6 IC_{50} value of ascorbic acid (ASA)

Absorbance of the blank	Concentration (µg/ml)	Absorbance of the extract	% of inhibition	IC_{50} value µg/ml
0.324	0.977	0.193	40.43	5.90
	1.953	0.175	45.99	
	3.906	0.186	42.59	
	7.813	0.139	57.10	
	15.625	0.098	69.75	
	31.25	0.068	79.01	
	62.5	0.024	92.59	
	125	0.015	95.37	
	250	0.006	98.15	
	500	0.005	98.46	

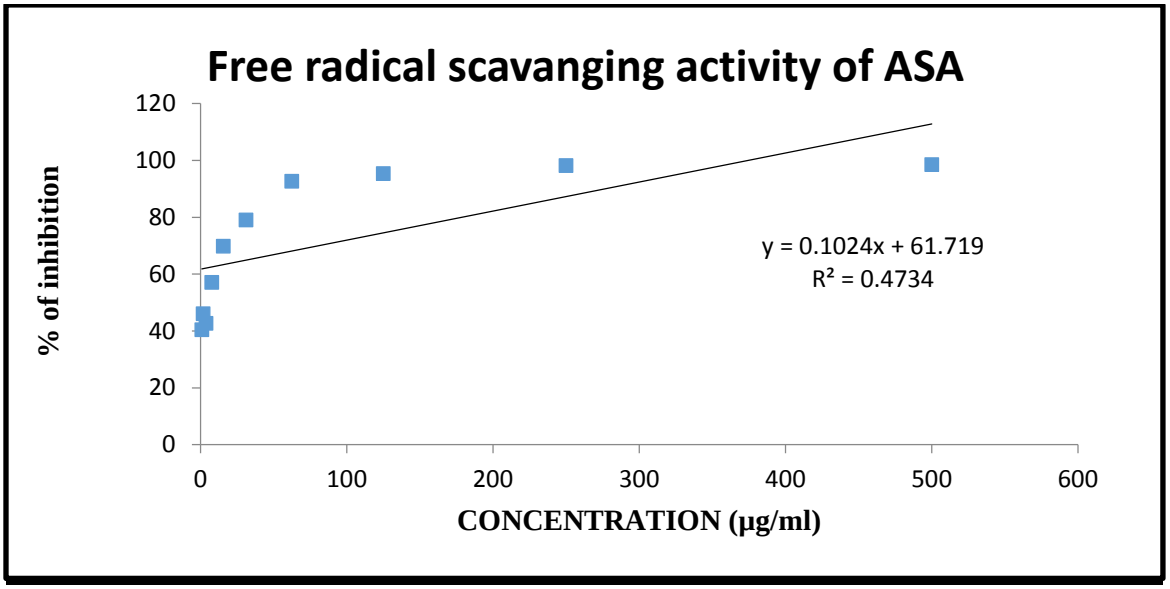

Figure 3.5 IC_{50} value of ascorbic acid (ASA)

Table 3.7 IC_{50} value of crude methanol extracts (CME)

Absorbance of blank	Concentration	Absorbance of the Extract	% of Inhibition	IC_{50} VALUE
0.324	0.977	0.590	-82.099	611.824
	1.953	0.558	-72.222	
	3.906	0.512	-58.025	
	7.813	0.480	-48.148	
	15.625	0.445	-37.346	
	31.25	0.420	-29.630	
	62.5	0.414	-27.778	
	125	0.406	-25.309	
	250	0.386	-19.136	
	500	0.228	29.630	

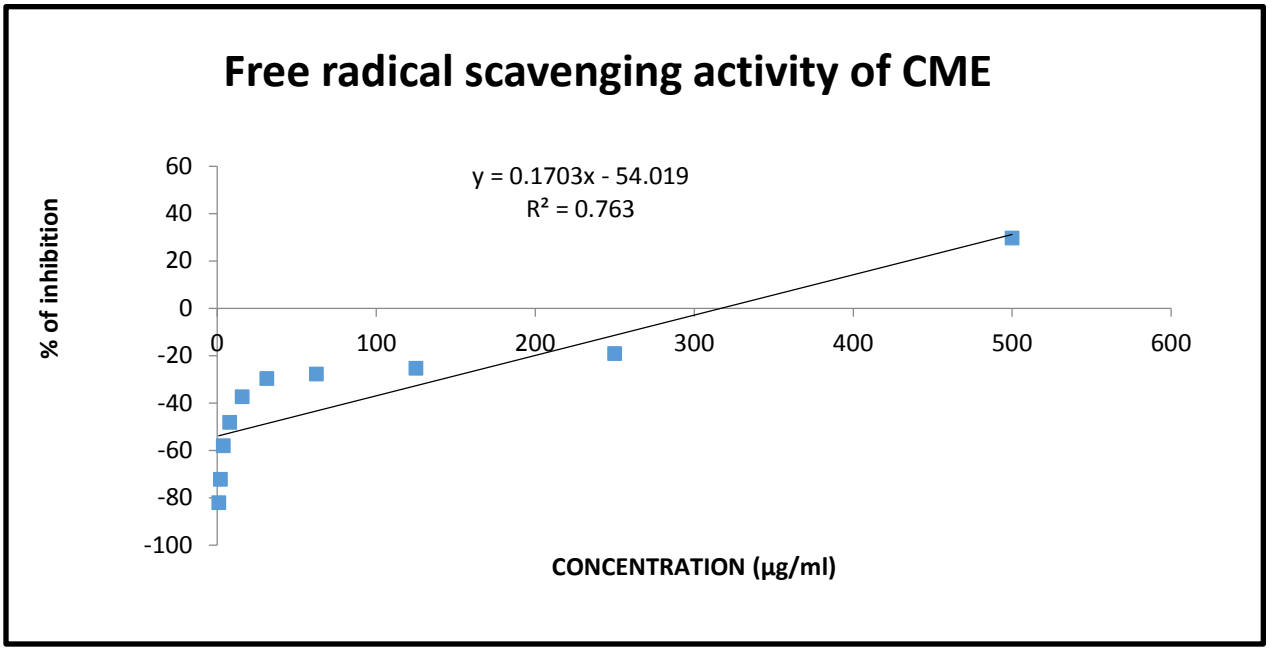

Figure 3.6 IC_{50} value of crude methanol extract (CME)

Table 3.8 IC_{50} value of crude acetone extracts (CAE)

Absorbance of blank	Concentration	Absorbance of the Extract	% of Inhibition	IC_{50}
0.324	0.977	0.512	-58.025	702.791
	1.953	0.476	-46.914	
	3.906	0.454	-40.123	
	7.813	0.432	-33.333	
	15.625	0.427	-31.790	
	31.25	0.415	-28.086	
	62.5	0.408	-25.926	
	125	0.393	-21.296	
	250	0.384	-18.519	
	500	0.237	26.852	

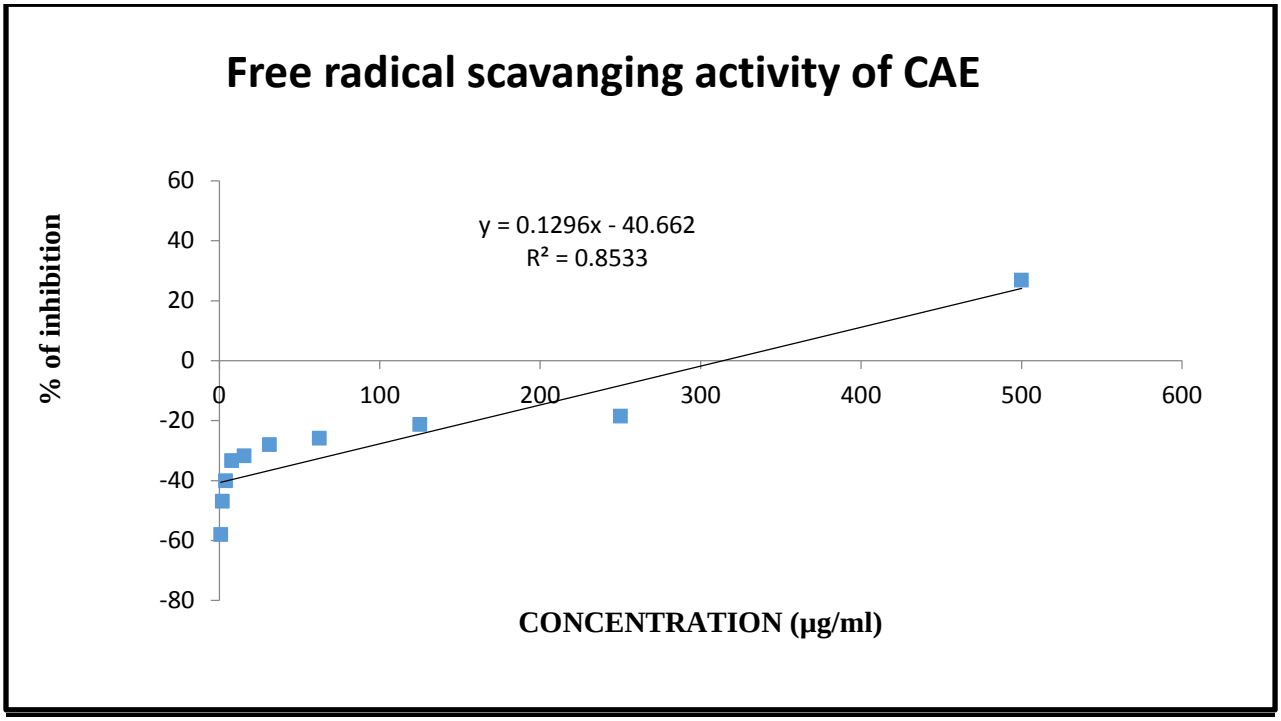

Figure 3.7 IC_{50} value of ascorbic acid (ASA)

3.4 Brine Shrimp Lethality Bioassay

Table 3.9 Test samples after serial dilution containing different concentration

Number of test tube	Concentration (µg/ml)
1	400
2	200
3	100
4	50
5	25
6	12.5
7	6.25
8	3.125
9	1.5625
10	0.7815

3.4.1 Result and discussion of Brine Shrimp Lethality Bioassay

The determination of the test sample's lethal concentration LC_{50} were obtained by plotting brine shrimp mortality percentage versus sample concentration logarithm. To gain the best fit-line the regression analysis curve data is used. The LC_{50} value of positive control group was found 0.451µg/mL and showed a remarkable result against the negative control group. The LC_{50} value of vincristine sulphate which is a positive control group was been compared with the sample extract's LC_{50} value.

Table 3.10 LC_{50} values of the samples

Test samples	Regression Line	R^2	LC_{50} value(µg/mL)
Vincristine sulphate (VS)	y = 30.8x + 60.64	0.972	0.451
Methanol Extract (ME)	y = 28.18x + 30.85	0.951	4.781
Acetone Extract (AE)	y = 8.656x + 84.20	0.386	0.108

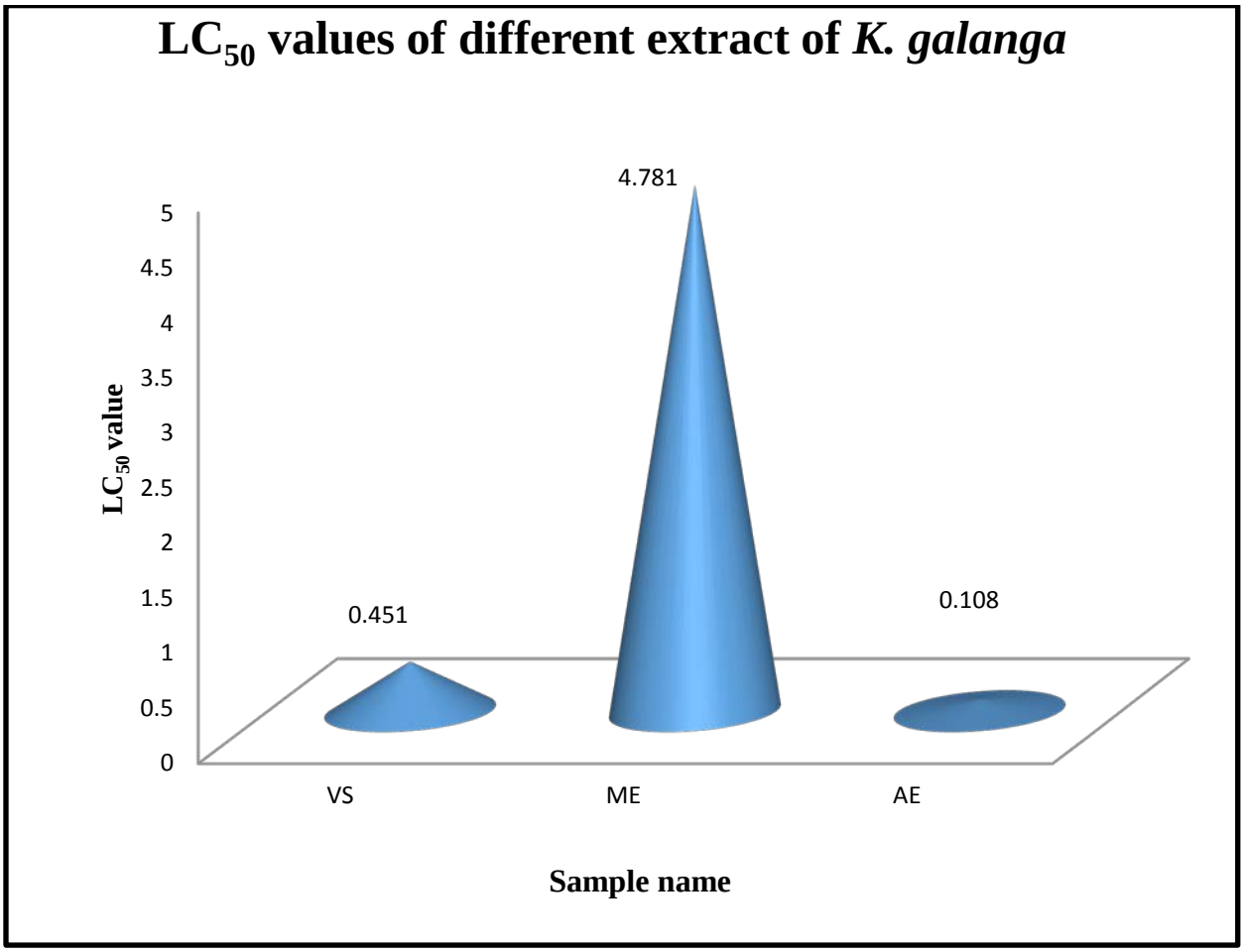

Figure 3.8 LC_{50} values of different extract of *K. galangal*

Table 3.11 Effect of positive control group (Vincristine sulphate) on shrimp nauplii

Concentration (µg/mL)	Log10 Concentration	Mortality percentage (%)	LC_{50} value (µg/mL)
0	0	20	0.451
0.0390	-1.4089	20	
0.078125	-1.1072	30	
0.15625	-0.8061	30	
0.3125	-0.5051	40	
0.625	-0.2014	50	
1.25	0.09691	70	
2.5	0.39794	80	
5	0.6989	80	
10	1.00	90	
20	1.3010	100	

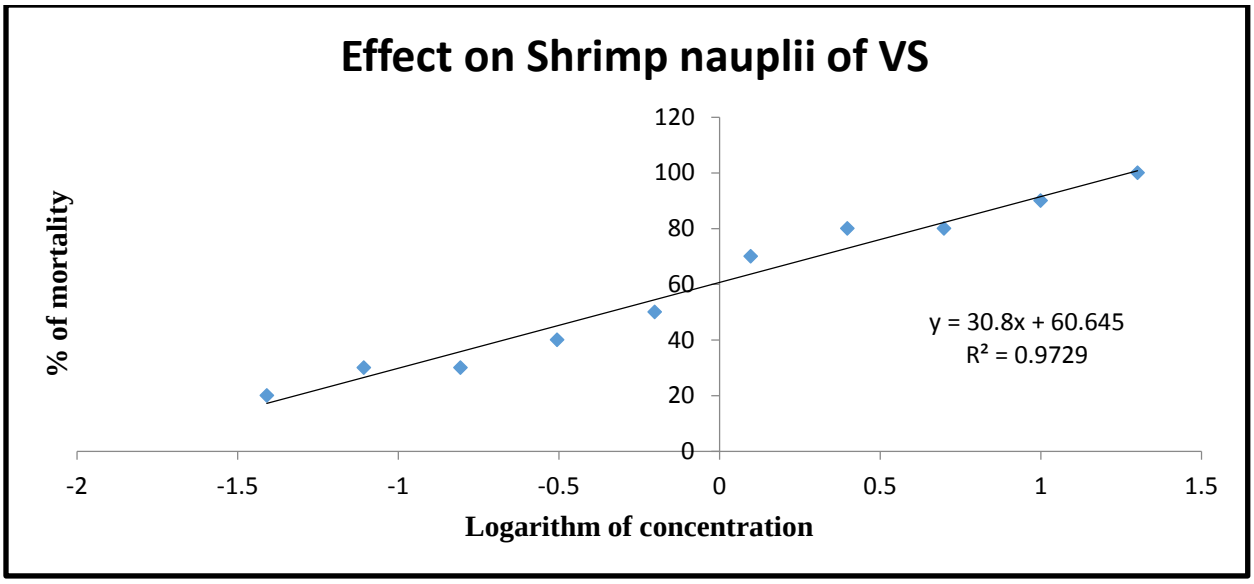

Figure 3.9 Plot of mortality percentage and predicted regression line of VS

Table 3.12 Effect of methanol extract on shrimp nauplii

Concentration (µg/mL)	Log10 Concentration	Mortality percentage (%)	LC_{50} value (µg/mL)
0.78	-0.1079	20	4.781
1.56	0.1931	30	
3.125	0.4948	50	
6.25	0.7958	60	
12.5	1.0969	70	
25	1.3979	70	
50	1.6989	80	
100	2	90	
200	2.301	90	
400	2.602	100	

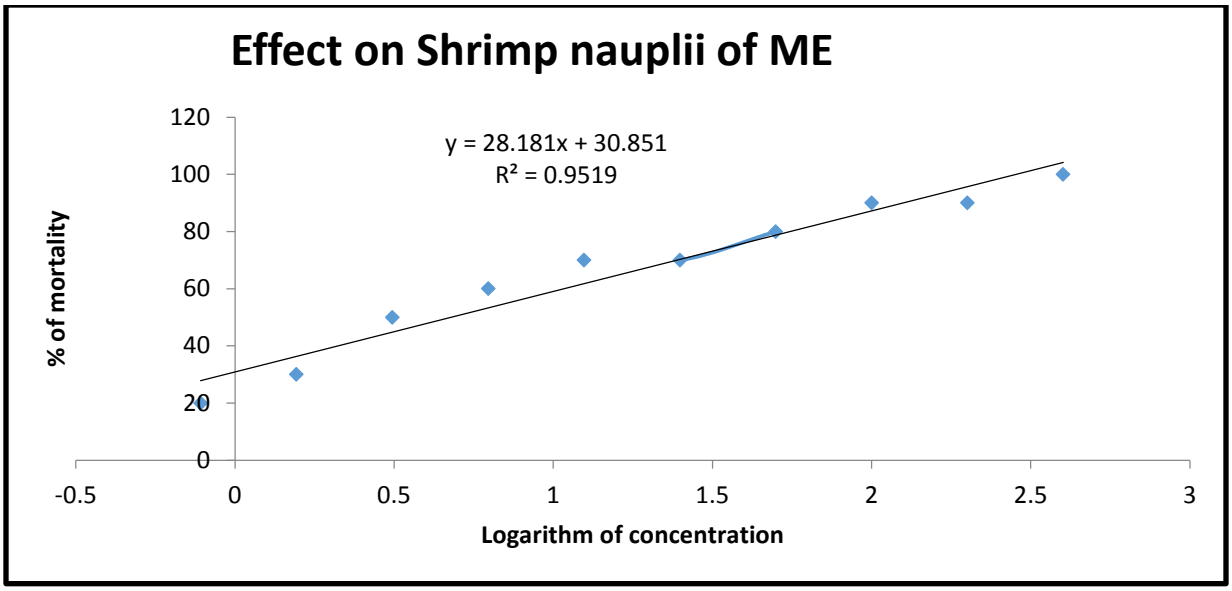

Figure 3.10: Plot of mortality percentage and predicted regression line of ME

Table 3.13: Effect of acetone extract on shrimp nauplii

Concentration (µg/mL)	Log10 Concentration	Mortality percentage (%)	LC_{50} value (µg/mL)
0.78	-0.1079	50	0.108
1.56	0.1931	70	
3.125	0.4948	80	
6.25	0.7958	80	
12.5	1.0969	90	
25	1.3979	100	
50	1.6989	100	
100	2	100	
200	2.301	100	
400	2.602	100	

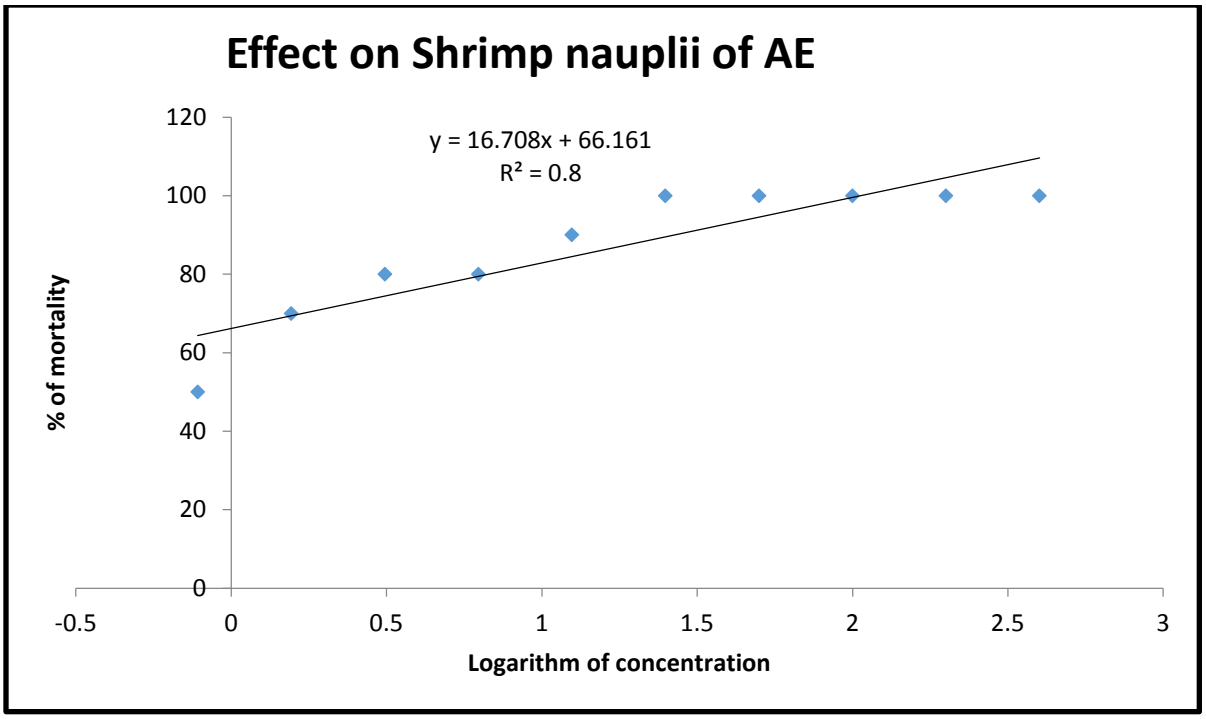

Figure 3.11: Plot of mortality percentage and predicted regression line of AE

Chapter four:

Conclusion

The plants *K. galangal* or aromatic ginger are already has gained the acceptances worldwide because of their medicinal activity, odor and tastes. The further and advanced study and research could improve and enhance their application in more broader and appropriate range. In this study, the various fraction of *K. galangal* has been used to investigate biological and phytochemical activity.

Here the most famous phytochemical investigation total phenolic content measurement has been done of the various extracts of the plant. The result shows that crude methanol extract (CME) (0.380 GAE/gm of dried sample) has the greater total phenolic content compared to the crude acetone extract (CAE) (-0.019 GAE/gm of dried sample).

Furthermore, to investigate biological activity the importance were given in the *in-vitro* antioxidant activities and cytotoxic effects of the various extracts of the plant. DPPH test and brine shrimp assay were performed to determine the IC_{50} and LC_{50} values respectively and were compared with the controls.

Chapter Five:

References

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Ahmed, A., Husain, A., Mujeeb, M., Khan, S. A., Aldrami, H. A. B. &Bhandari, A. (2015).Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of *Piper nigrum Linn*fruits.*AsianPacific Journal of Tropical Biomedicine*, 5(2) , 101-107.

Ahn YJ, Kim NJ, Byun SG, Cho JE, Chung K. Larvicidal activity of Kaempferiagalangal rhizome phenylpropanoids towards three mosquito species. Pest Management Science 2008; 64(8):857–862.

American Journal of Food and Nutrition, 2015 3 (3), pp 64-68. doi: 10.12691/ajfn-3-3-1.

Anonymous.The Wealth of India, Volume V; CSIR, New Delhi, 1959, 314.

Arambewela L, Perera A, Thambugala R, Wijesundera RLC, Gunatileke J. Investigations on Kaempferiagalanga. Journal of the National Science Foundation of Sri Lanka 2000; 28(3):225-230.

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 Kaempferiagalanga L. SciHort 2005; 104:113-120.

Choochote W, Kanjanapothi D, Panthanga A, Taesotikul T, Jitpakdi A, Chaithong U et al. Larvicidal and repellent effects of *Kaempferiagalanga*. South east Asian J Trap Med Public Health 1999; 30:470-476.

Dake GN, Manoj PS. Bacterial wilt of *Kaempferiagalanga L*. caused by Pseudomonas solanacearum (Smith) Smith. Journal of Spices and Aromatic Crops 1995; 4(2):159.

Fontenot, B. (2011). Antioxidants Explained: Why these coumpounds are so important,*The Atlantic Monthly Group*. Retrieved from http://www.theatlantic.com/health/archive/2011/10/antioxidants-explained-why-these-compounds-are-so-important/247311/

Flora of North America Expertise Network, Zingiberaceae of North America Update, database

Chapter Five: References

(version 2010)

Ghani A. Medicinal plants of Bangladesh: Chemical constitutions and use. Asiatic Soc Bangladesh, 1998, 290- 291.

Gunathilake HAJ, Arambewela L, Ratnayake H, Rajapakse S. Feasibility of growing medicinal plants in coconut lands of the wet zone of Sri Lanka.Cocos 2000; 14:87-96.

Hasslberger, B. (2007). Networking for a better future: Synthetic antioxidants can harm your health. *Organic consumer association.* Retrieved from https://www.organicconsumers.org/scientific/synthetic-antioxidants-can-harm-your-health

Hirschhorn HH. Botanical remedies of the former Dutch East Indies (Indonesia).J Ethanopharmacol 1983; 72:123- 156.

Hofmann, A., Ratsch, C., Schultes, R., Plants of the Gods: Their Sacred, Healing, and Hallucinogenic Powers. Rochester: Healing Arts Press, 1992.

International Research Journal of Biological Science, 2(11), 74-77. ISNN: 2278-3202

Jagadish PC, Raghu Chandrashekhar H, Vinod Kumar S, Latha KP. Potent selective cytotoxic activity of Kaempferiagalanga rhizome against cancer cell cultures. International Journal of Pharma and Bio Sciences 2010; V1:1-5.

John Timbrell, Introduction to Toxicology, Third release, p3-4.

Kanjanapothi D, Panthong A, Lertprasersuke N, Taesotikul T, Rujjanawate C, Kaewpinit D. Toxicity of crude rhizome extract of Kaempferiagalanga L. (ProhHom) J Ethnopharmacol 2004; 90(2-3):359–365.

Kaushita B, LekhyaPriya C, BhaskaraRao KV. HPLC analysis and antioxidant activities of hydroethanolic leaf extract of Kaempferiagalanga Linn. International Journal of Pharm Tech Research 2015; 7(2):422-431.

Kirtikar KR, Basu BD. Indian Medicinal Plants, volume IV. In: Blatter E, Caius JF, Mahaskar KS (eds.), Lalit Mohan Basu, Allahabad, India, 1996, 2422-2423.

Kiuchi F, Nakamura N, Tanda Y, Konaa K, Yoshimura H. Studies on crude drug effective on visceral larva margins II. Larvicidal principles in Kaempferia rhizome.Chem Pharm Bull 1988; 36:412-415.

Kosuge T, Yokoto M, Sugiyama K, Satio M, Iwata Y, Nakkura M et al. Studies on anticancer principles in Biota orientalis and Kaempferiagalanga. Chem Pharm Bull 1985; 33:5565-5567.

LalithaBai EK, Augustin A. Performance of Kacholam (Kaempferiagalanga L.) under varying levels of organic and inorganic fertilizers. Proceedings of 10th Kerala Science Congress, Kozhikode, 1998, 254-256.

Larsen K, Ibrahim H, Khaw SH, Saw LG. Gingers of Peninsular Malaysia and Singapore. Natural History Publications, Borneo, 1999, 135.

Maheswarappa HP, Nanjappa HV, Hegde MR. Effect of planting material, plant population and organic manures on growth components and yield of galangal (Kaempferiagalanga) when grown as an intercrop in coconut garden. Indian Journal of Agricultural Science 2001; 71(3):183- 186.

Marinova, G. &Batchvarov, V. (2011).Evaluation of the methods for determination of the free radical scavenging activity by DPPH.*Bulg. J. Agric. Sci.,* 17, 11-24

Mokkhasmit M, Swatdimongkol K, Satrawah P. Study on toxicity of Thai Medicinal Plants. Bull Dept Med Sci 1971; 12:36-65.

Naresuan University Journal, 12(2), 13-18.

National Plant Data Center, NRCS, USDA. Baton Rouge, LA 70874-4490 USA. http://plants.usda.gov, The PLANTS Database, database (version 4.0.4),1996

Nisha MS, Sheela MS. Effect of green leaf mulching for the management of root knot nematode in kacholam. Indian Journal of Nematology 2002; 32(2):211-212.

Chapter Five: References

Noro T, Miyase T, Kuroyanagi M, Ueno A, Fukushima S. Monoamine oxidase inhibitor from the rhizomes of Kaempferiagalanga L. Chem Pharm Bull 1983; 31:2708- 2711.

Olowa, L. F. &Nuneza, O. M. (2013).Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from IliganCity, Philippines.

Perry, M., and Metzger J., "Medical Plants of Eastand Southeast Asia. Attributed Properties and Uses", *Cambridge, MIT Press,* p. 442, 1980.

Pisutthanan, S., Plianbangchang, P., Pisutthanan, N., Ruanruay, S. &Muanrit, O. (2004).Brine Shrimp Lethality Activity of Thai Medicinal Plants in the Family Meliaceae.

Plant Resources of South-East Asia No 12(1): Medicinal and Poisonous plant 1

Raghavan TS, Venkatasubban KR. Cytological studies in the family Zingiberaceae with special reference to chromosome number and cytotaxonomy. Proc. Indian Academy of Science Series B. 1943; 17:118-132.

Rahman MM, Amin MN, Ahmed T, Ali MR, Habib A. Efficient plant regeneration through somatic embryogenesis from leaf base derived callus of Kaempferiagalanga L. Asian J Plant Sci 2004; 3(6):675- 678.

Rajagopalan A, Gopalakrishnan PK. Growth, yield and quality of Kaempferiagalanga L. as influenced by planting time and type of seed material. Agricultural Research Journal of Kerala 1985; 23(1):83-89.

Ratsch, Christian., The Encyclopedia of Psychoactive Plants: Ethnopharmacology and its Applications. Rochester: Park Street Press, 1998.

Ramachandran K. Chromosome numbers in Zingiberaceae. Cytologia 1969; 34:213-221.

Ravindran PN, Balachandran I. Underutilized medicinal spices (II). Galanga (Kaempferiagalanga L.). Spice India 2005; 18(1):22-35 58. Revikumar K, Ved DK. Red Listed Medicinal Plants of Conservation Concern in Southern India, 1st edition, 2000, 212-214.

Retrieved From*VanWyk, Ben-Erik (2005). Food Plants of the World. Portland, Oregon: Timber Press, Inc.* ISBN 0-88192-743-0

Ridtitid, W., Sae-wong, C., Reanmongkol, W., and Wongnawa, M., "Antinociceptive activity of the methanolic extract of *Kaempferiagalanga* Linn. In experimental animals",*JEthnoharmacol*, 118(2): 225-230, 2008.

Sadimann JA. Little known Asian Spice and medicinal plant (Kaempferiagalanga L.).Pharmazie 1992; 47:636- 639.

Sass, C; Iles, WJ; Barrett, CF; Smith, SY; Specht, CD (21 January 2016). "Revisiting the Zingiberales: using multiplexed exon capture to resolve ancient and recent phylogenetic splits in a charismatic plant lineage." *PeerJ*. 4: e1584. *doi*:*10.7717/peerj.1584*. *PMID 26819846*.

Shanbhang Tara V, Chandrakala S, Sachidananda A, Kurady BL, Smita S, Ganesh S. Wound healing activity of alcoholic extract of Kaempferiagalanga in Wistar rats. Indian J PhysiolPharmacol 2006; 50:384-390.

Sirirugsa, P., "Thai Zingiberaceae: Species diversity and their uses", *Pure &Appl Chemistry*, 70(11): 4-5, 1997.

Spearing JK, MahantyHK.The relationship of the African species of Kaempferia to those found in Asia.Abs. Xth International Botanical Congress, Edinburgh, 1964.

Sudibyo RS. The contents of volatile oil isolated from Kaempferiagalanga rhizomes mass spectroscopic approach. MajalahFarmasiIndones 2000; 11:142-149.

Sulaiman MR, Zakaria ZA, Duad IA, Hidayat MT. Antinociceptive and anti-inflammatory activities of the aqueous extract of Kaempferiagalanga leaves in animal models. J Natural Medicines 2008; 62:221-227.

Thomas J, Joy PP, Mathew SS, Skaria BP. Indigenous less known essential oils perspective.Pafai Journal 1998; 20(1):13-20.

Tewtrakul S, Yuenyongsawad S, Kummee S, LatthyaAtsawajaruwan L. Chemical components and biological activities of volatile oil of Kaempferiagalanga Linn. Songklanakarin J SciTechnol 2005; 27:503-507.

Umar, M.I., Asmawi, M.Z.B., Sadikun, A., Altaf, R., an Iqbal M.A., "Phytochemistry and medicinal properties of *Kaempferiagalanga L.* (Zingiberaceae) extracts", *Afr. J. Pharm. Pharmacol*, 5(14): 1638-1647, 2011.

Umar, M.I., Asmawi, M.Z., Sadikun, A., Atangwho, I.J., Yam, M.F., Altaf, R., and Ahmed, A., "Bioactivity-Guided Isolation of Ethyl-*p*-methoxycinnamate, an Anti-inflammatory Constituent, from *Kaempferiagalanga* L. Extracts", *Molecules*, 17: 8720-8734, 2012.

United States Department of Agriculture, Retrived from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=Zingiberaceae

Voogelbreinder, Snu, Garden of Eden: The Shamanic Use of Psychoactive Flora and Fauna, and the Study of Consciousness. SnuVoogelbreinder, 2009.

Wang, X. & Quinn, P. J. (1999).Vitamin E and Its Function in Membranes.*NCBI*, 38(4), 309-336.

Wong KC, Qng KS, Lim CL. Composition of the essential oil of rhizome of Kaempferiagalanga L. Flavour and Fragrance J 1992; 7:263-266.

Wood TH. Biogeography and the evolution of the Zingiberaceae.Zingiberaceae Workshop, Prince of Songkla University, Hat Yai, Thailand, 1991, 6-7.

Xue Y, Chen H. Study on the anti-carcinogenic effect of three compounds of Kaempferiagalanga L. XeiShen Yan Jiu, 2002; 31(4):247–248, 251.