"Studies on the medicinal properties of Alpinia Galanga"

A Dissertation

Submitted By

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To

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In partial fulfilment of the Requirement for the

Award of the Degree of

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Under the guidance of

DR. TABARAK MALIK

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INDIA'S LARGEST UNIVERSITY

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DISSERTATION TOPIC APPROVAL PERFORMA

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1. Studies on the medicinal properties of Alpinia Galanga
2. To study the antioxidant and anticancerous property of alpinia galanga
3. To study the activity of alpinia galanga as ~~different~~ antioxidant and anticancerous.

OK

Signature of Supervisor 4889

PAC Remarks:

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13083

APPROVAL OF PAC CHAIRPERSON: Signature: Date:

*Supervisor should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)

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*One copy to be submitted to Supervisor.

ABSTRACT

The current study evaluates different medicinal properties of Alpinia Galanga, i.e. antioxidant via DPPH, superoxide anion radical scavenging activity and metal ion chelating assay, antidiabetic via α-amylase and α-glucosidase activity, antimicrobial potential via agar well diffusion method with ethanolic, methanolic, ethyl acetate, butanolic and aqueous extracts of rhizome and leaf part of this plant. The results of phytochemical analysis of different extracts indicated the presence of alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, glycosides and cardiac glycosides in different extracts of plant. The methanolic extract of rhizome and aqueous extract of leaf found to have highest scavenging activity via DPPH. In metal chelating assay, aqueous extract of rhizome was found to exhibit highest activity and methanol of rhizome showed highest activity in superoxide anion radical scavenging assay. Methanol of rhizome also showed highest inhibition against alpha-amylase and alpha-glucosidase in both assays. Futher experiment showed that the highest inhibition zone diameter was exhibited by ethanol extract of leaf against *E.coli* and butanol extract of rhizome against *B.subtilis*.

CERTIFICATE

This is to certify that SHRADDHA SHARMA bearing registration no.11012064 has completed M.tech dissertation **entitled "Studies on the medicinal properties of *Alpinia galangal*"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of the dissertation has been submitted to any other University or Institute for the award of any degree or diploma. The dissertation is fit for submission and the partial fulfilment of the condition for the award of Master of technology in Biotechnology.

Date: 5-may-2015

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Last but not the least, I would like to thank my beloved parents for their endless and unwavering love which were undeniably the bedrock upon which the past years of my life ***have been built. I'm also grateful for the strength and enthusiasm which they have given m****e to achieve success in every spheres of my life. Without their care and support, the present work would have been a mere dream.*

SHRADDHA SHARMA

DECLARATION

I hereby declare that the dissertation entitled **"Studies to the medicinal properties of alpinia galangal"** submitted for M.Tech degree is entirely my original work and all ideas and references have been duly acknowledged. It does not contain any work for the award of any other degree or diploma.

Date: 5-may-2015

Shraddha Sharma
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Chapter-1

INTRODUCTION

Plants are the most dominant organisms on the earth. They compensate with the nature and are most adapted to it fully. Due to different adaptation, the plants have developed different dominating properties. Due to these different beneficial properties the plants are used as traditional medicines from ancient times. Nature has been source for different medicines or the origin of large number of present efficient drugs. These medicinal plants contain the constituents in a balanced way and the extracted products, formulated into different medicines (may or may not be toxic). The broad range of plant products or derived ones like dietary supplements, phyto-chemicals, pro-vitamins, etc that help in maintaining the good health are nowadays considered as the active ingredients of plants. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents. This thesis gives detail on research into the chemistry and pharmacology of plant, Alpinia Galanga from Zingiberaceae family, in the terms of its use as antioxidant, antidiabetic, antimicrobial agents.

1.1 Plants as medicine

Since ancient time, various types of medicines have been provided by the plants for the benefit of humans. The oldest known document containing details of medicinal plants and their uses, written 4800 years ago, describes about 360 plants.

Alpinia galangal is extremely used in medical field. Many homeopaths and herbalist prescribe the galangal oil for its medicinal uses. Digestion process can be improved, vomiting and constipation can also be reduced by regular consumption of galangal. This remedy is very effective for the inflammation and ulcers of stomach. Blood circulation (mostly in hands in feets), nutrient and oxygen supply can be improved by the use of galangal. Congestion like respiratory problems and breathing rate can be improved bad better regulated by galangal.

Very effective remedy to treat cough and cold is a mixture of galangal and lime juice. Additionally, for bad breath galangal powder is used as a mouth freshener.

Despite a number of therapeutic advancements, AIDS remains a major global health concern, for which therapeutics must be generated urgently. Acetoxychavicol acetate (ACA), a small molecular compound isolated from the rhizomes of *Alpinia galanga*, inhibited reverse transport at a low concentration by binding to chromosomal region maintenance 1 and accumulating full-length HIV-1 RNA in the nucleus, resulting in a block in HIV-1 replication in peripheral blood mononuclear cells. Additionally, ACA and didanosine acted synergistically to inhibit HIV-1 replication.

1.2 Phytochemicals

Phytochemicals are wide variety of chemical compounds that occur naturally in plant, that are non-nutritive chemicals with disease protective properties. Thousands of phytochemicals have been identified by scientists, that may affect human health, but only small fraction have been studied yet.

Many of the health problems like heart disease, cancer, diabetes, high blood pressure etc. can be prevented by promoting these phytochemicals. Potential carcinogens are the substances that cause cancer might be prevented with the help of phytochemicals by blocking the action of carcinogens on target tissue or by acting on such cells to suppress the development of cancer. Consumption of more fruits, vegetables and other foods containing such chemicals is adviced by many experts to minimize the risk of cancer.

There are several major groups of phytochemicals:

Flavonoids are plant chemicals found in a various range of grains, fruits and vegetables. The studies are carried out on these to determine that whether the chronic diseases like cancer and heart diseases can be prevented. The The actions of the female hormone estrogen can be copied by isoflavones found in licorice, foods and supplements such as garbanzo beans, soy products, red clover, and lignans found in whole grains and flaxseed. The substances like estrogen extracted from these plants are known as phytoestrogens. The major role is played

by these phytoestrogens against some hormone-dependent cancers in the development of and protection.

Phenols are also the large family of phytonutrients having family members more than 2,000. Culinary herbs contain single phenolic unit in abundance. The herbs that contain phenol includes apiole, rosmarinol, carvacrol (oregano). They all are used as food preservatives from long time. Various activities are performed by phnols in humans such as antioxidants, antifungals and antiseptics.

Saponins are found in legumes, having their highest concentration in soybeans. Various experimental studies showed that it contains many medicinal activities such as anticancer, immunostimulatory, cholesterol lowering properties etc. regulation of cell proliferation, antioxidant effects and immune modulations results in the anticancerous properties. Rduction in high cholesterol level was observed in the animals when daidzein, soy protein was given to them.

1.3 Antioxidants

Free radicals are highly reactive chemicals that have the potential to harm cells. When there is either gain or loss of an electron in an atom or molecule, it creates free radicals. They play a major role in various cellular processes as they are form naturally in the body. Sometimes, they may be hazardous to the body at high concentrations and thus may damage all the major components of cells (DNA, cell membranes, proteins). Due to such damage in the body can play major role in developing cancer and other conditions.

Chemicals that interact with and neutralize free radicals are known as antioxidants and the cellular damage caused by free radicals can be prevented by them, so they are also known as "free radical scavengers."

Antioxidant required to neutralize free radicals are synthesized by body itself, thus these are known as endogenous antioxidants. Rest of the antioxidant that body needs are fulfilled by the external sources, that are known as exogenous antioxidants, which are commonly called dietary antioxidants. Rich source of these exogenous antioxidants are fruits, vegetables, and grains.

1.4 Plant profile

Botanical name: Alpinia galnga

Synonym: Languas galangal, Maranta galanga L.,A.galanga willd

Common names: Galanga Root, Greater Galanga, Siamese Ginger, kulnjan, rasna

Family: Zingiberaceae

Habitat : Cultivated in south east asia

Description:

The plant is perennial herb, aromatic, up to 2.5m high with 1.0m spread; with thick fragrant tuberous rootstocks, resembling the scent of ginger, from which the new shoots sprout in the spring. Leaves linear- lanceolate, alternating, produced on reedy stems with fringed borders 15-25x3-6 cm ; white sheath, long ligule rounded . Inflorescence racemose, tubular flowers bisexual, produced in pendulous panicles on a separate leafless peduncle, with large ovate white bracts, perianth tremors , one stamen and three carples, red in color with whitish pink or yellow labellum, The fruits are red berries; seeds obtusely angular and aromatic.

Traditional & Medicinal Uses :

Alpinia galangal was known to the ancient Indians, and has been found to be spread its use in the West since the Middle ages. The rhizome is pungent, carminative, stimulant, appetizer, aphrodisiac, carditonic. Rhizomes and seeds used against rheumatism, bronchial catarrh, bad breath, and ulcers, whooping colds in children, throat infections, to control incontinence of urine, remedy for fever , dyspepsia, sexual impotency, diabetes; rhizome paste for skin problems and acnes, used in manufacturing as a fermenting agent. . Alpinia species show promise as anti-fungals, hypotensives, enhancers of sperm count and motility. Anti-tumor and anti-dementia effects have been observed in rodents. It is widely used in many countries as a food flavoring and a spice.

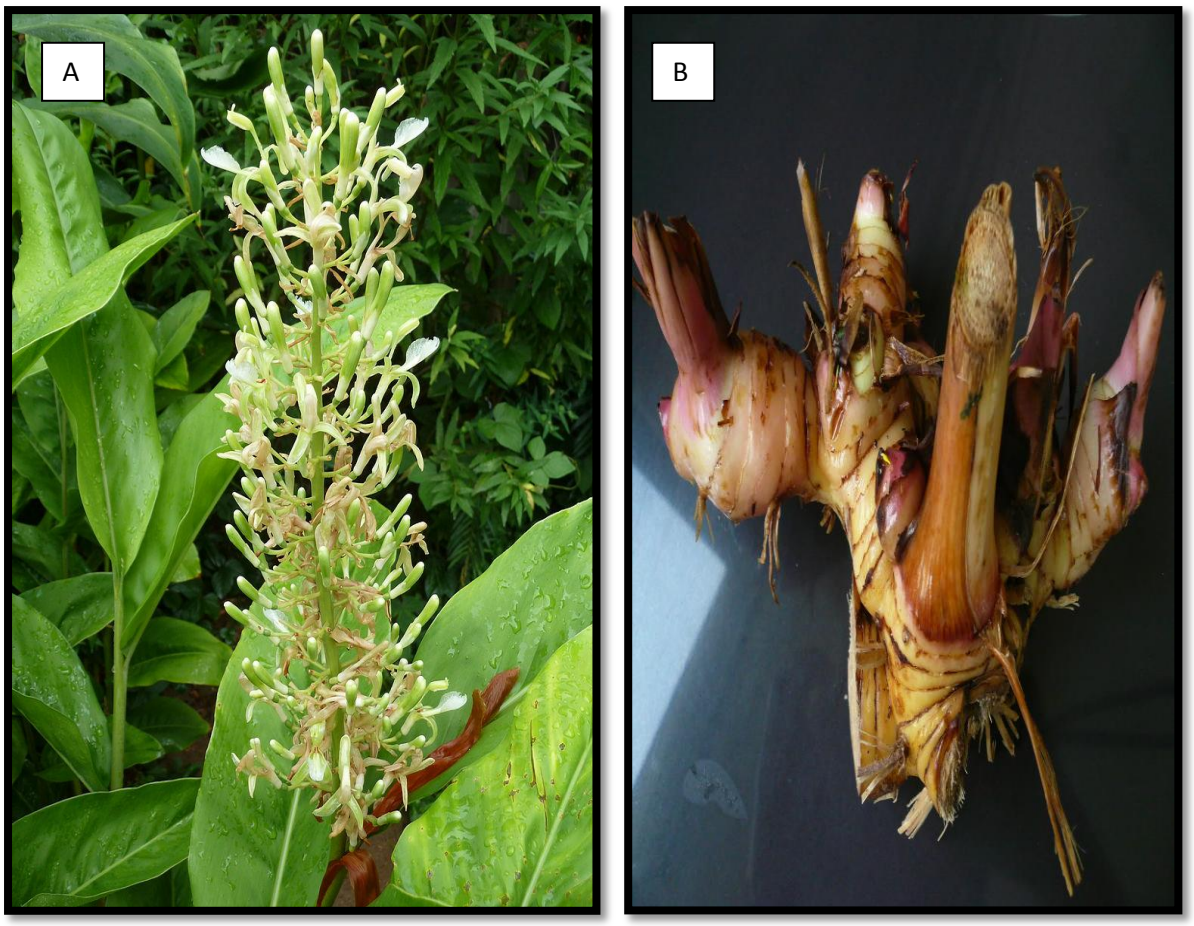


Fig.1: a) arial part of plant b) rhizome of A.galanga

Classification**:**

Kingdom: Plantae

Order: Zingiberales

Family: Zingiberaceae

Subfamily: Alpinioideae

Tribe: Alpinieae

Genus: *Alpinia*

Species: *A. galanga*

Chapter-2

REVIEW OF LITERATURE

In **1972, M. A. Al-Yahya, S. Rafatullah, J. S. Mossa** studied on Gastric Antisecretory, Antiulcer and Cytoprotective Properties of Ethanolic Extract of Alpinia galanga Willd in Rats. The effect of Alpinia galanga extract has been studied on experimentally induced gastric ulcers in rats. The ethanolic extract of A. galanga at a dose of 500mg/kg, significantly reduced the intensity of gastric mucosal damage induced by pyloric ligation and hypothermic restraint stress in rats. It produced a significant decrease in gastric secretion in pylorus ligated rats and a highly significant cytoprotective effect against 80% ethanol-, 0.6 M HC1-, 0.2 M NaOH- and 25% NaCl-induced cytodestruction. Pretreatment with the extract significantly prevented hypothermic stress-induced gastric wall mucus depletion. These findings suggest that a significant antisecretory and cytoprotective action of A. galanga may be responsible for its antiulcer activity.

In **1990, Al-Yahya, M. A. *et* al** reported some of the medicinal properties from the ethanolic extract of alpinia galangal as antiulcer, gastric antisecretory and cytoprotective properties. in rats. In Arabian and unani systems, rhizomes of A.galanga are widely used to treat stomach disorders. The ethanolic extract also significantly reduced gastric secretion and showed marked cytoprotective activity; it is suggested that these properties may be responsible for the antiulcer activity of Alpinia galangal.

In **1992, S. Qureshi , A. H. Shah , A. M. Ageel** studied on Toxicity Studies on Alpinia galanga and Curcuma longa. Rhizomes of *Alpinia galanga* and *Curcuma longa* with their ethanolic extracts were used for studies of acute (24 h) and chronic (90 days) oral toxicity in mice. 0.5, 1.0, 3.0 g/kg of acute dosages body weight and 100mg/kg/day of chronic dosage as the extract was used. With all changes including external morphological, spermatogenic and hematological, the vital organ and body weight were also recorded. As comparion with controls, any significant mortality was not observed between during this investigation. The animals treated with *A. galanga* were heavy weighed as in control groups but no weight was gained by the animals treated with C. Longa treated animals. There were significant changes

in heart and lungs weights by treating them with *C. longa* upon chronic treatment. It was studied that the animals treated with *A. galangal* showed a significant rise in level of RBC and the animals treated with C.longa showed a significant fall in the WBC and RBC levels as compared to the controls, by hematological studies. The extract of A.galanga treated male mice showed the increased sperm motility and sperm counts and gain in weights of sexual organs. No spermatotoxic effects was showed by both extracts.

In **1994, S.QURESHI *et al*** studied on the effect of alpinia galangal treatment on cytological and biochemical changes induced by cyclophosphamide (CP) in mice. In this experiment, the mice was administerd with the ethanolic extract of alpinia galangal. This treatment caused the mitodepression of bone marrow but did not affected the number of micronucleated polychromatic erythrocytes (PCE) in bone marrow cells. Cyclophosphamide induced the micronucleated PCE and this effect was also reduced by the treatment of A.galangal without altering the cytotoxicity. CP treatment caused some of the biochemical changes that were also inhibited by this treatment.

In **1996, Hiroyuki Haraguchi , Yoshiharu Kuwata , Kozo Inada** studied on Antifungal Activity from *Alpinia galanga* and the Competition for Incorporation of Unsaturated Fatty Acids in Cell Growth. An antimicrobial diterpene was isolated from *Alpinia galanga* in the screening for potentiators of phytochemical antibiotic action. The structure was elucidated by spectral data and identified as (*E*)-8β, 17-epoxylabd-12-ene-15,16-dial. Diterpene *1* synergistically enhanced the antifungal activity of quercetin and chalcone against *Candida albicans*. Antifungal activity of *1* was reversed by unsaturated fatty acids. Protoplasts of *C. albicans* were lysed by *1*. These results suggest that antifungal activity of *1* is due to a change of membrane permeability arising from membrane lipid alternation.

In **1997, C. R. Achuthan and Jose Padikkala** studied on alpinia galangal and kaempferia galangal for hypolipidemic effect. For the treatment of various inflammatory diseases, obesity, and diabetes mellitus, the widely used medicines are from the rhizome of both *Alpinia galanga* and *Kaempferia galangal*. Their research and studies evaluated that in vivo hypolipidemic action was found in the ethanolic extract from the rhizome of these plants. The levels of serum and tissues of total cholesterol, phospholipids, triglycerides were found to be lowered by oral dose of the extracts of both *A. galanga* and *K. Galangal and* the serum levels of high density lipoproteins(HDL) in high cholesterolwas significantly increased on

fedding white wistar rats over a period of 4 weeks. The results indicated the presence of these plants in the treatment of various lipid disorders especially atherosclerosis.

In **1998, Kikue Kubota, Kae Nakamura, and Akio Kobayashi**, they discussed about the odorous components of rhizome of alpinia galangal. They identified four isomers of acetoxycineoles, -2- and 3-acetoxy-1,8-cineoles (in trans or cis form), as the aromatic components in the selected plant. The structures of these aromatic compounds were confirmed by the comparision between the retention indices of Gas chromatography and the mass spectrometry with those of synthesized compounds. The highest concentration among all four isomers was found to be of *trans*-3-acetoxy-1,8-cineole and also it was seemed to have strongest qualitative effect on the characteristic flavour of alpinia galanga. The (*trans* and *cis*)-2 isomers was found to exhibit woody and sweet aromas odor feature respectively, while the (*trans* and *cis*)-3 isomers showed sweet floral and camphoraceous aromas respectively.

In **1999, Yang Xion Gen et al** isolated and identified 1'-acetoxychavicol acetate from rhizome of Alpinia galanga. Gas Chromatography Analysis was used for the identification.

In **2002, M.S. Akhtar, M.A. Khan, and M.T. Malik** worked on the hypogylycaemic activity of alpinia galangal rhizome and its extracts in rabbits. Effects of *Alpinia galanga* rhizome on blood glucose levels were investigated in this study. The blood glucose was lowered in normal rabbits by dosing them powdered rhizome and its methanol and aqueous extracts. A significant decrease in blood glucose in the rabbits was also produced by Gliclazide. No effect was shown in the lowering the blood glucose level by *A. galanga* and its methanol and aqueous extracts in alloxan-diabetic rabbits. The comparison of gliclazide with hypoglycaemic effect of *A. galanga* in normal rabbits was performed. The high level of certain minerals were found to be contained by rhizome. From behavioural and acute toxicity studies, any abnormal behaviour or any signs of toxicity was not found in rabbits even at higher dose of extracts. Thus from this studied the conclusion was that, reduction in blood glucose level was produced by *A. galanga* in normal rabbits.

In **2005, T. Juntachote, E. Berghofer** studied on Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal. The aims of this work were to assess the influence of concentration, heat treatment, and pH value on antioxidant activity of ethanolic extracts obtained from Holy basil (*Ocimum sanctum Linn*) and Galangal (*Alpinia galanga*).

The antioxidative properties were evaluated. Good heat stability (80 °C, 1 h) showed the ethanolic extracts of Holy basil and Galangal. Holy basil extracts had high antioxidative stability at neutral and acidic pH, whereas higher antioxidative stability were showed by Galangal extracts at neutral than at acidic pH ranges. Antioxidant activity of both extracts at neutral pH was higher than at acidic pH ranges. Strong superoxide anion scavenging activity, Fe2+ chelating activity, and reducing power in a concentration-dependent manner were exhibited by holy basil and Galangal extracts. Antioxidant activity of both extracts correlated well with reducing power. Furthermore, ethanolic extracts of Holy basil and Galangal acted as radical scavenger and also as lipoxygenase inhibitor.

In **2005, Somia Khattak, Saeed-ur-Rehman and Hamid Ullah Shah** studied on the biological effects of indigenous medicinal plants Curcuma longa and alpinia galangal. The excellent phytotoxic activity against *Lemna minor* was shown by the ethanolic extracts of *Curcuma longa* and *Alpinia galangal*. Good antifungal activities against *Trichophyton longifusus* was also found in these extracts (65% and 60%, respectively), while toxicity with LD_{50} of 33 and 109 µg/ml was found in the brine shrimp lethality bioassay. These extracts were found quite inert in antibacterial bioassay, while for insecticidal activity the extract from *C. longa*, was tested, was also found to be devoid of any activity.

In **2006, S. Phongpaichit, V. Vuddhakul, S. Subhadhirasakul and C.Wattanapiromsakul** studied on the Evaluation of the Antimycobacterial Activity of Extracts from Plants Used as Self-Medication by AIDS Patients in Thailand. Chloroform, methanol, and water extracts from medicinal plants used as self-medication by AIDS patients in Thailand were evaluated for their antimycobacterial activity using the microplate Alamar blue assay. The crude extracts exhibited antimycobacterial activity with minimum inhibitory concentrations (MICs) of 0.12–1000 µg/ml. The chloroform extract of *Alpinia galanga*. rhizomes and *Piper chaba*. fruits had strong inhibitory effects with MIC values of 0.12 and 16 µg/ml, respectively. The active compounds, 1′-acetoxychavicol acetate from *Alpinia galanga*. and piperine from *Piper chaba*. had MIC values of 0.024 and 50 µg/ml, respectively.

In **2006, Ying Ye and Baoan Li,** isolated ACA from the rhizomes of alpinia galangal, collected from the region of china. 100 gm of alpinia galangal was extracted with methanol. Silica-gel column chromatography was used to purify the extract and purified extract was eluted with chloroform/methanol (1 : 1) and n-hexane or ethyl acetate at different

concentration. The reverse-phase HPLC was then used to purify n-hexane/ethyl acetate 5 : 1 fraction to obtain a pure compound of 1.5 gm. Then by comparing the spectral data of mass and nuclear magnetic resonance with that of compounds reported, the chemical structure of this pure compound was identified as ACA.

In **2006, Thongchai Taechowisan, Asawin Wanbanjob and Pittaya Tuntiwachwuttikul** worked on the identification of streptomyces species, an endophyte in alpinia galangal and isolation of actinomycin D. Firstly, isolation of 120 endophytic actinomycetes from roots of lpinia galangal were done. On the basis of their morphology and composition of amino acids of whole cell extracts, these endophytes wre identified. 82 *Streptomyces* species, 11 *Nocardia* species, and 3 *Microbispora* species and 2 *Micromonospora* species were those isolated that were classified. During subculture, some of them(14) were lost and remained 8 isolates were not classified. They observed that *Colletotrichum musae* and*Candida albicans* were strongly inhibited by the strain that was identified as endophytic *Streptomyces* sp. Tc022. This endophyte was then cultured, the agar was extracted with organic solvent and purification of the extract was performed on a silica gel column to give a major component, on the basis of spectroscopic data, that was further identified as actinomycin D. The antifungal activity was shown by component Actinomycin D against *Colletotrichum musae* and *Candida albicans* with the MIC of 10 and 20 mg ml^{-1}, respectively.

In **2007, L.G. Korkina** studied on the phenylpropanoids as naturally occurring antioxidants: from plant defence to human health. Phenylpropanoids belong to the largest group of secondary metabolites produced by plants, mainly, in response to biotic or abiotic stresses such as infections, exposure to ozone, wounding, pollutants, UV irradiation and other environmental conditions. Antioxidant and free radical scavenging properties are considered to be the molecular basis for the protective action of phenylpropanoids in plants. Major active (biological) components of human diet, spices, aromas, propolis, traditional medicine and wines, beer, essential oils. Natural and synthetic phenylpropanoids attracts todays interest for various medicinal use as an antioxidant, anti-virus, wound healing, anti-inflammatory, anticancer, and UV screens. They are used as active natural ingredients for cosmetic and perfume industries. The metabolic pathways of phenylpropanoid biosynthesis in plants and the mechanism of phenylpropanoid-mediated plant defense are described in this paper.

In **2007, Siddharthan Surveswaran, Yi-Zhong Cai, Harold Corke , Mei Sun** studied on Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. ABTS, DPPH and FRAP assays were used to assess the total antioxidant capacities of 133 Indian medicinal plant species sampled from 64 families, and Folin–Ciocalteu assay were used to measure their total phenolic contents. These species exhibited a broad range of antioxidant activities were exhibited by these species by ABTS assay varying from 0.16 to 500.70 mmol TEAC/100 g DW. The antioxidant activity values similarly varied with the DPPH and FRAP assays. Total antioxidant capacities and phenolic contents (R = 0.89–0.97) were found to have significant and positive linear correlations in between them that indicates that in tested medical plants, the phenolics that were the dominant antioxidant constituents. Preliminary identification of the major phenolic compounds from 83 selected medicinal plants by reversed-phase HPLC revealed phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, and quinines. Very high levels of hydrolysable tannins swas shown by the fruit of Terminalia chebula, pericarp of Punica granatum and gall of Rhus succedanea, and very high levels of catechin and epicatechin in addition to tannins were showedby the gum of Acacia catechu. Major phenolics in many of the medicinal plants were identified for the first time (e.g., Euphorbia lathyrus, Ipomoea turpethum, and Picrorrhiza kurroa). This systematic investigation of a large number of Indian medicinal plants proved important for understanding their chemical constituents and functionality in Ayurvedic medicine, and contributes to the search for natural sources of potent antioxidants.

In **2008, L.F. Wong, F.S. Lianto** and other members studied on the antioxidant and tyrosinase inhibition properties from the leaves and rhizomes of ginger species. The leaves of 26 ginger species belonging to nine genera and three tribes were screened for the total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC). For 14 species, TPC and AEAC of rhizomes were also assessed. The leaves and rhizomes of eight species were compared for its ferrous ion-chelating (FIC) abilities. Analysis on the five species of *Etlingera* out of 26 was done for tyrosinase inhibition activity on leaves of five species of were done. The leaves showed the highest TPC and AEAC. Out of 14 species, leaves were found to be shown higher TPC and/or AEAC than in rhizomes. Leaves of *Etlingera elatior* and*Etlingera maingayi* were found to have values of seven to eight times higher than those of rhizomes. Higher values of FIC ability was shown by leaves than in rhizomes of six out of the eight species. Alpinia galangal was found to have most outstanding which was more than 20 times higher than that of rhizomes. The strongest

tyrosinase inhibition activity was shown by the leaves of *E. Elatior* of the five species of *Etlingera*, followed by leaves of *Etlingera fulgens* and *E. maingayi*. In comparision to positive control, the values of their inhibition activity were significantly higher. Besides promising tyrosinase inhibition ability, high antioxidant activity and antibacterial properties were also shown by the leaves of these three *Etlingera* species.

In **2008, Pornpimon Mayachiew and Sakamon Devahastin studied** studied on the antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. They investigated on the antimicrobial and antioxidant activities of Indian gooseberry and Alpinia galangal extracts. Agar disc diffusion and agar dilution are the two different methods that were used to evaluate the antimicrobial activities of the given plant extracts against Staphylococcus aureus. 13.97 and 0.78 mg/ml are the respective minimum inhibitory concentration (MIC) values that were found in the Indian gooseberry and galangal extracts and 13.97 and 2.34 mg/ml are the respective minimum biocidal concentration (MBC). 86.4% and 70.3% are the respective percentage calculated by the b-carotene bleaching method for the antimicrobial activities of Indian gooseberry and galangal extracts. The Folin–Ciocalteu method was used to determine the total phenolic contents of Indian gooseberry and galangal extracts that was found to be 290.470.7 and 40.970.2 mg/g plant extract (in GAE), respectively. 1,8-cineole (20.95%), b-bisabolene (13.16%), b-caryophyllene (17.95%) and b-selinene (10.56%) were identified as the main compounds by the GC–MS analysis. Many other compounds that were present in the Indian gooseberry extract were detected by the use of high-performance liquid chromatography (HPLC) with UV detection.

In **2009, Kumari, AV Anita Gnana, R. Suraj** and other members worked on the In vitro antimicrobial evaluation of Curcuma Longa, Zingiber officinale, and Alpinia galangal extracts as natural food preservatives. In this paper, studies were done on the antimicrobial activity of these three plants against the common food borne bacteria such as salmonella entriditis, staphylococcus, escherischia coli, clostridium perfringens by using agar disc diffusion method. Antibacterial and antifungal properties was shown by all extracts. Maximum inhibition zone of $p<0.001$ was shown by methanol extracts. Zingiber officinale and Curcuma Longa showed greater activity than that of alpinia galangal.

In **2009, Nopparat Mahae and Siree Chaiseri** made some efforts to determine the antioxidant activity from the different extracts of alpinia galangal. Galangal was extracted by using 50% ethanol and two other samples from water extract and essential oil were compared with that of ethanolic extract. The activity was confirmed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) methods. The highest DPPH free radical scavenging ability as well as highest ORAC value was shown by ethanolic extract when compared to water extract and essential oil.

In **2009, S.B. et al reported** reported the isolation of galangoflavonoid from the rhizomes by column chromatography and eluted with ethyl acetatemethanol to yield galangoflavonoid.

In **2010, Wei-Yea Hsu, Amarat Simonne and Alexandra Weissman** worked on the antimicrobial activity of alpinia galangal flowers. Investigation was done on the effects of different drying methods and solvent types on the antimicrobial activity of flowers, the potential antimicrobial activity of flower of alpinia galangal against *Shigella, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella* were also investigated by using the agar disc diffusion method. The oven dried ethanol extract was found to be most effective extract from the flower of galangal against S. aureus, with about 26-31 mm of inhibition zone and the minimum inhibitory concentration (MIC) ranging from 0.352–0.547 mg/mL. No such antimicrobial activity was observed on *E. coli* and *Salmonella* by flower extract. Oven-dried samples extracted with ethanol showed the highest overall antimicrobial activity with 8.84mm inhibition zone and 1.457 mg/mL MIC. In contrast, overall antimicrobial activity of freeze-dried samples extracted with ethanol was lowest with 7.05 mm inhibition zone and 2.470 mg/mL MIC. This is the first report that described the work against Grampositive bacteria *S. aureus* to check antimicrobial activity of flowers of alpinia galangal.

In **2010, Kiranmayee Rao and Bhuvaneswari Ch** worked on the antibacterial activity from the crude extracts of alpinia galangal. Extracts of alpinia galangal was prepared with methanol, acetone and diethyl ether, and by using agar well diffusion method they were evaluated against different pathogens viz. *Bacillus subtilis* MTCC 2391, *Escherichia coli* MTCC 1563, *Staphylococcus aureus, Enterobacter aerogene, Pseudomonas aeruginosa* MTCC 6642, *Enterobacter cloacae, Streptococcus epidermis, Enterococcus faecalis,, Salmonella typhimurium and Klebsiella pneumoniae*. Using macrodilution method,

minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of all the extracts were determined. Excellent activity by methanolic extracts was shown towards all the pathogens with MBC and MIC values that ranges from 0.08–2.56 mg/ml and 0.04–1.28 mg/ml, respectively. Compounds that can be responsible for its broad spectrum activity, like 59.9% of 5-hydroxymethyl furfural, 15.65% of 1,8 cineole, 57.6% of benzyl alcohol and 9.4% of methylcinnamate, 8.5% of 3-phenyl-2-butanone and 8.9% of 1,2 benzenedicarboxylic acid, were obtained by the GC–MS analysis of methanol extracts. So, for new drug discoveries or new drug development *alpinia galanga* can be quite resourceful.

In **2011, Afzal Unnisa and Thahera D. Parveen** studied on the acute toxicity of extract form the rhizomes of alpinia galangal. The experiment was employed on the group of male wister rats which were given with oral dose of methanolic extract and observed after 72 hrs for mortatlity, behavioural changes, convulsions, any toxic symptoms. No effect was observed under single dose of extract but on providing higher dose, they observed some changes.

In **2012, Debasis Bhunia and Amal Kumar Mondal** discussed about various species of alpinia along with alpinia galangal. In this paper, it is observed that the alpinia is an important medicinal plant used in eastern India and mostly used by the people's of tribe of Paschim Medinipur district in various domestic and for different medicinal purposes. The main part of this plant that is used is rhizome that is an important antimicrobial agent and a digestive stimulant and also has various others medicinal properties. Three most important species are like Alpinia galanga L., Alpinia calcarata Rosc. and A. allughas Rosc. are three most important species that contain various important chemical constituents such as flavones: alpinin, galangin,and 1'S'-1'-acetoxychavicol acetate, cinol, methyl cinnamate, cyanidin, amino acids, etc. These are the most active species against these four bacterial pathogens are, Salmonella typhi, Staphylococcus aureus, Escherichia coli and Bacillus cereus, that uses agar well diffusion method. The highest diameter of inhibition zone is found to be 15mm in rhizome of Alpinia galanga L. and A. allughas Rosc. Significant role against Staphylococcus aureus was observed by all selected species.

CHAPTER-3

SCOPE OF STUDY

Antioxidants are the natural substance that may prevent various types of cell damage by inhibiting the oxidation of other molecules. Free radicals are product of these oxidation reaction that can form chain reaction. In cell, when chain reaction starts, it causes damage to cell, that is prevented by antioxidants. Antioxidant plays a major role in maintaining health by preventing various diseases like Alzheimer's disease, cancer, diabetes, cardiovascular diseases, rheumatic disorder, mascular degeneration, DNA damage and ageing.

Antioxidants properties have been found to be present in phytochemicals, that are necessary for curing such diseses. Presence of such phytochemicals in Alpinia galangal can be used to reduce oxidative stress caused by reactive oxygen species (ROS). This study provides scientific intuition to determine the antioxidant, antidiabetic and antimicrobial properties of A.galanga. On the basis of different research, it will be revealed that whether or not *Alpinia galangal* can be used as a natural antioxidant, antidiabetic and antimicrobial agents against infections and/or diseases.

CHAPTER-4

AIMS & OBJECTIVES OF STUDY

Plants from Zingiberaceae family contains rich source of phytochemicals and have been reported to have various medicinal properties such as anticancerous, antioxidant, antimicrobial, antidiabetic. The present study will focus on A.galanga with following objectives-

1. Phytochemical analysis
2. Determining the antioxidant properties of *A.galanga*
3. Determining the antidiabetic activity of *A.galanga*
4. Determining the antimicrobial activity of *A.galanga*

CHAPTER-5

MATERIALS AND METHOD

5.1 Instrumentation

Grinder

Water bath

Incubator

Glass ware (conical flasks, beakers, etc.)

Digital balance

Spectrophotometer

Laminar Air Flow

Oven

Glassware (Petri plates, test-tubes, micropipettes, beakers)

5.2 Chemicals and reagents

- **Extract preparation**

  Ethanol, Methanol, Butanol, Ethyl acetate.

- **Phytochemical screening**

  Hydrochloric acid (HCl), Dragendorff's reagent, Dilute ammonia solution, Aluminium chloride, Sodium nitrite, Sulphuric acid, Dilute sodium hydroxide, Fehling solution A & B, Chloroform, n-Hexane, Ferric chloride, Millon's reagent, Hydrated copper sulphate, Molisch reagent, Folin- ciocalteu reagent, Gallic acid, Sodium carbonate, Quercetin.

- **Antioxidant activity**

  Ammonium-thiocyanate, 1,1-diphenyl 2-picrylhydrazyl (DPPH), Trichloroacetic acid, Ascorbic acid, Nitro Blue tetrazolium (NBT), Riboflavin, Butylated Hydroxy

Toluene, FeCl2, Ferrozine, Riboflavin solution, EDTA solution, methanol and nitro-blue tetrazolium (NBT) solution, DMSO, Phosphate buffer

- **Antidiabetic activity**

  Sodium phosphate buffer, α-amylase solution, starch solution, dinitrosalicylic acid (DNS) reagent, phosphate buffer, α-glucosidase solution, pnitrophenyl-α-D-glucopyranoside (PNPG), NaCO3

- **Antibacterial activity**

  Nutrient agar, E.coli (MTCC 40), Bacillus subtilis (MTCC 441), DMSO, Gentamycin disc

5.3 Methodology

It includes all the steps starting from the collection of sample.

5.3.1 Collection of plant samples

The whole plant of *Alpinia galanga* were collected from a local area of Imphal, Manipur. The plant samples were identified by the taxonomist at D.M. College of Science, Imphal, Manipur. The rhizome and leaf part of the plant *A.galanga* were separated and cleaned in running tap water followed by distilled water and dried in shade. The parts under study i.e leaf and rhizome of *Alpinia* were then ground in a coarse powder using pestle and mortar and preserved in air-tight containers.

5.3.2 Preparation of plant extracts

The coarsely powdered rhizome and leaf of *A.galanga* were subjected to cold extraction process by soaking them in different solvents (methanol, ethanol, butanol, ethyl acetate and water) in the ratio 1:10 at room temperature for 48 hours, according to the method described by **Pratap Chandran R. *et.al*.(2013).** Each sample was filtered through Whatman no. 1 filter paper. The solvents were evaporated on rotary evaporator. Solid extracts were collected and stored. Stock solution was prepared by dissolving different solvent extracts in DMSO for 5mg/ml and used for the estimation of different assays.

Table1. Characteristic of crude extracts of *A.galanga*

Sr.no	solvent	Sample Alpinia galangal		odour
		leaf	root	
1	Ethyl acetate	Light yellow	Brown	Characteristic
2	Ethanol	Dark green	Light brown	Characteristic
3	Methanol	Dark brown	Yellowish	Characteristic
4	Butanol	Yellow	Dark yellow	Characteristic
5	Water	Dark green	Dark brown	Characteristic

5.3.3 Phytochemical Screening

Qualitative :

The following phytochemical tests were carried out on the different extracts of A. galangal to determine the active constituents. These tests were performed to detect the presence of various secondary metabolites such as alkaloids, tannins, saponins etc. in the plant under investigation.

Test for Alkaloids

2ml of plant extracts were boiled with 5ml of 2% HCl on steam bath. This solution was divided into two parts(1ml each). To one part, wagner's reagent was added dropwise. Formation of reddish brown ppt indicated for the presence of alkaloids. To the other part, dragendorff's reagent was added, resulting into the red ppt which indicated the presence of alkaloids.

Test for Terpenoids

To 2ml of each of the plant extracts, 2ml of glacial acetic acid was added. The solution was shaken and to this 1ml of conc. H_2SO_4 was carefully added from the side of the test tube. The formation of blue green rings indicated the presence of terpenoids.

Test for Flavonoids

To 1ml of plant extract, few drops of 10% NaOH. To this few drops of conc. HCl was added. A yellow colouration that disappears on standing indicated the presence of flavonoids.

Test for Tannins

To 1ml of extract, 1ml of 1% lead acetate was added. Allowed it to stand for 15 minutes. The formation of yellow ppt indicated the presence of tannins.

Test for Cardiac Glycosides (Keller-Killiani Test)

1ml of plant extract was treated with 2ml of glacial acetic acid containing few drops of $FeCl_3$. The formation of brown color ring indicates the presence.

Test for Phenols (Ferric chloride test)

1ml of aqueous extract was dissolved in 2ml of distilled water, and a few drops of 10% ferric chloride solution were added. A bluish black or green colour was produced indicating the presence of phenols.

Test for steroids

10ml of chloroform was mixed with 1ml of aqueous plant extract. To this solution, 10ml of conc H_2SO_4 was added carefully from the side of the test tube. Formation of red color in the upper layer and yellow with green fluorescence in sulphuric acid layer indictes the presence of steroid.

Test for glycosides

5ml of dilute sulphuric acid was added to 1ml of aqueous extract and allowed it to boil for 15 minutes in water bath. After cooling mixture of Fehling solution A & B was added to it and left for 15 minutes. Formation of red color ppt indicates its presence.

Quantitative:

Total Flavonoid Content Determination

Colorimetric aluminium chloride method was used for the determination of the total flavonoid content of the sample extracts (**Mervat et al., 2009; Nabavi et al.,2008**). 0.1ml $AlCl_3$ (10%), 0.1ml potassium acetate (1 M) and 2.8ml distilled water were added in a test tube. The mixture was shaken vigorously. The mixture was incubated for 30 minutes and absorbance at 415nm was recorded. A standard calibration graph was plotted at 415nm using quercetin as standard with the concentration of 1mg/ml. The total flavonoid content of extracts were determined and expressed as quercetin equivalence, QE (mg/g).

Total flavonoid content = (absorbance of test/absorbance of standard) x concentration of standard.

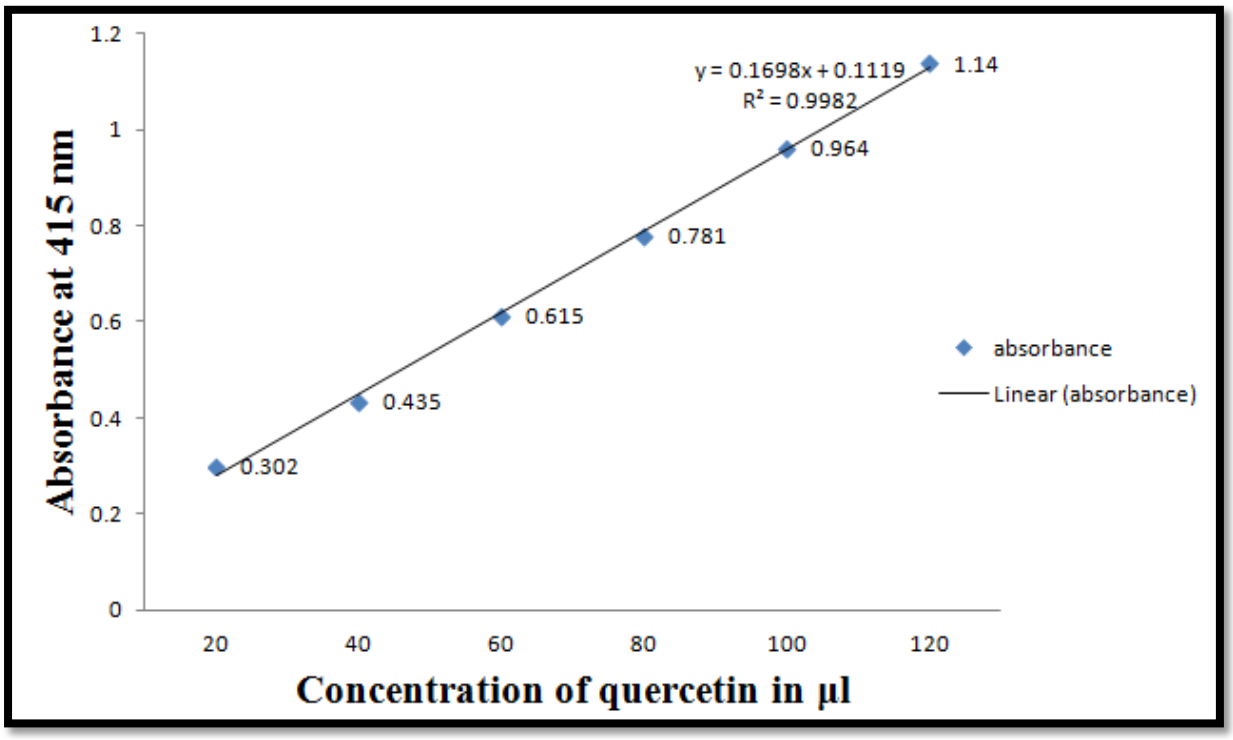


Fig2: Quercetin calibration curve

Total Phenolic Content Determination

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method (**K. Wolfe *et al.*, 2003**). 0.5ml 0f gallic acid was mixed with 2.5ml of 1/10 aqueous dilution of Folin-Ciocalteu reagent. After 5 minutes the reaction was neutralized with 2 ml saturated sodium carbonate (7.5%). It was incubated for 120 minutes at room temperature. The absorbance was measured at 765 nm. Gallic acid was used as a standard in concentration of 1 mg/ml and expressed in terms of gallic acid equivalence, GAE (mg/g).

Total phenolic content = absorbance of test/absorbance of standard) x concentration of standard.

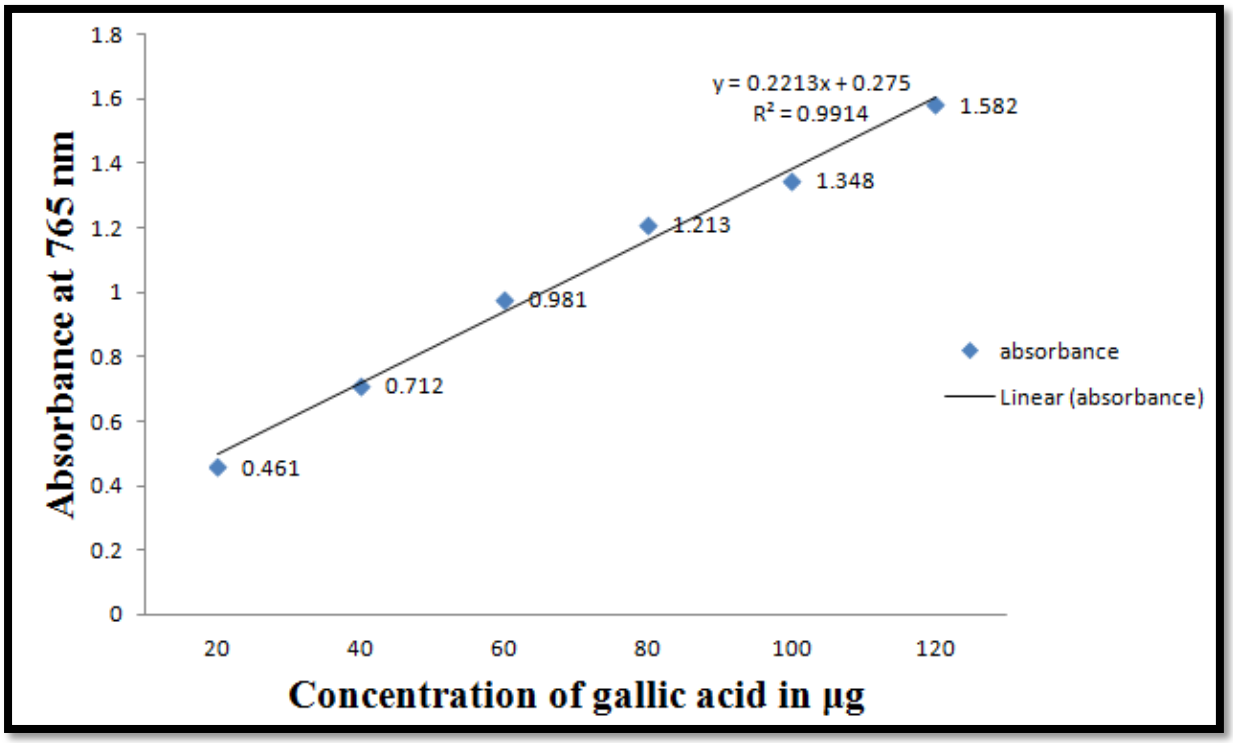


Fig3: Gallic acid calibration curve

5.3.4 Antioxidant activity

DPPH Radical Scavenging Activity

The DPPH assay method has been the most accepted test for evaluating the free radical scavenging activity of any compound. The radical scavenging effect of extracts were

determined by DPPH free radical scavenging assay **(Tepe *et al.*, 2005)**. Here, 1ml of different concentration solvent extracts of leaf and rhizome were mixed with 1ml of DPPH solution (0.002% with different solvents) in separate tubes. The tubes were incubated in dark for 30 minutes at room temperature. The absorbance of each tube was measured at 517nm in a spectrophotometer. The absorbance of control (1ml of DPPH+1ml of different solvents) was also noted. Ascorbic acid was used as reference standard. The radical scavenging activity of each concentration of leaf and rhizome extract was calculated using the formula:

Radical scavenging activity (%) = (Ac-At/Ac) x100, where Ac is absorbance of control and At is the absorbance of extract/standard. The concentration of extract required to scavenge 50% of free radicals (IC50) was calculated.

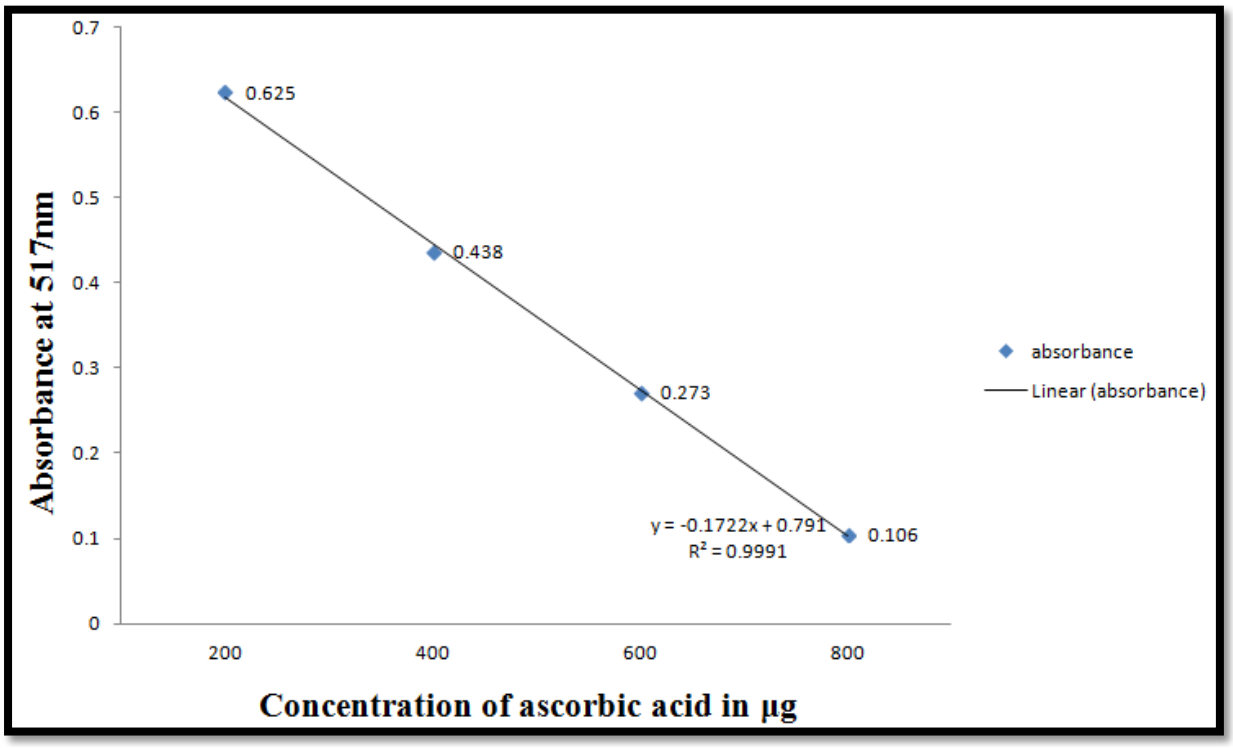


Fig.4: Ascorbic acid standard curve

Metal chelating activity

The chelation of ferrous ions by extracts was estimated by method of Dinis et al. **(Dinis *et al.*, 1994)** with little modification**.** Briefly, 100 µl of 2 mM FeCl2 was added to different concentration of extracts. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–$Fe2^{+}$ complex formation was calculated as:

[(A0- As)/ As] X 100, where A0 was the absorbance of the control, and As was the absorbance of the extract/ standard. EDTA was used as positive control.

Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of the all extracts based on the method described by Liu *et al*. (1997) with slight modification was carried out. 100 µl riboflavin solution (20µg), 200µl EDTA solution (12mM), 200µl methanol and 100µl nitro-blue tetrazolium (NBT) solution (0.1mg) were mixed in test tube and reaction mixture was diluted upto 3ml with phosphate buffer(50mM). The absorbance of solution was measured at 590nm using phosphate buffer as blank after illumination for 5 minutes. This is taken as control. Different concentrations of plant extracts were taken and diluted with DMSO. To each of these add 100µl riboflavin, 200µl EDTA, 200µl methanol and 100µl NBT was mixed in test tube and further diluted upto 3ml with phosphate buffer. Absorbance was measured after illumination for 5 minutes at 590nm. Decreased absorbance of the reaction mixture indicates increased super oxide anion scavenging activity. The % inhibition of super oxide anion generation was calculated using the following formula:

SOD scavenged (%) = Ac-As /Ac x 100 Where 'Ac' is the absorbance of the control and 'As' is absorbance of sample.

5.3.5 Antidiabetic Activity

α-Amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty. A total of 250µL of extract (1.25 – 10 mg/ml) was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes were pre-incubated at 25^{o}C for 10mins, after which 250 µL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals. The reaction mixtures were incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 min and cooled to room temperature. The content of each test tube was diluted with 5 ml distilled water and the absorbance measured at 540 nm in a spectrophotometer. A control was prepared using the same procedure except that the extract was replaced with distilled water. The α-amylase inhibitory activity was calculated as:

% Inhibition = {(Ac – Ae)/Ac}100

where Ac and Ae are the absorbance of the control and extract, respectively.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was assessed by the standard method (Dong et al., 2012), with slight modifications. Briefly, a volume of 60 µl of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37 ºC for 20 min. After pre-incubation, 50 µl of 5 mM pnitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 ºC for another 20 min. Then the reaction was stopped by adding 160 µl of 0.2 M NaCO3 into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

Inhibition Percentage (%) = A0-A1/ A0×100 Where, ACO is absorbance of the control and at is absorbance of the sample.

5.3.6 Antibacterial Activity

Agar well diffusion

Antibacterial activity was determined by the agar-well diffusion method. Nutrient agar was used as media. Plates were dried for 30 min at 37°C before plating. Then pouring was carried out. Thereafter 6 mm diameter wells were punched in the agar plates. Spreading of *E.coli* and *Bacillus subtilis* was done. Different extracts (100µl) of the leaves and rhizome of plant were added to the wells. Gentamycin disc was used as positive control (10µg/disc) and DMSO (100µl) was used as negative control. The plates were then incubated at 37°C for 24 h. After incubation the antibacterial activity was evaluated by measuring the inhibition zone diameter observed. Each test was performed thrice and the average of the results was taken.

CHAPTER-6

RESULT & DISCUSSION

6.1 Extraction

After the extraction process, the stock solution was prepared for different solvents and were kept in the plastic cap flasks. Following are the images that shows the leafs and rhizomes extracted with different solvents.

Fig.5: Filteration through whatmann paper after extraction

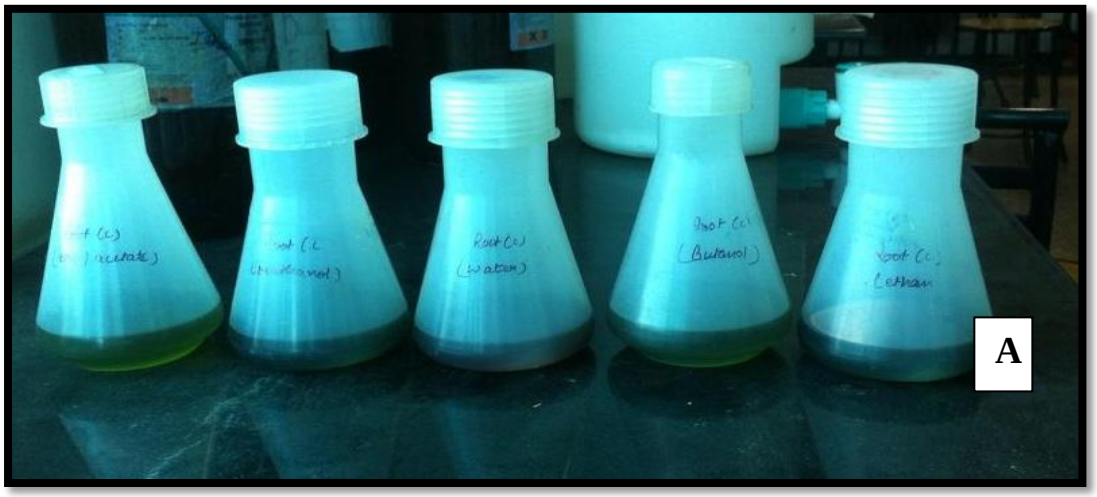


Fig.6: A and B is showing different stock solution of extracts of rhizome and leafs respectively.

6.2 Phytochemical screening

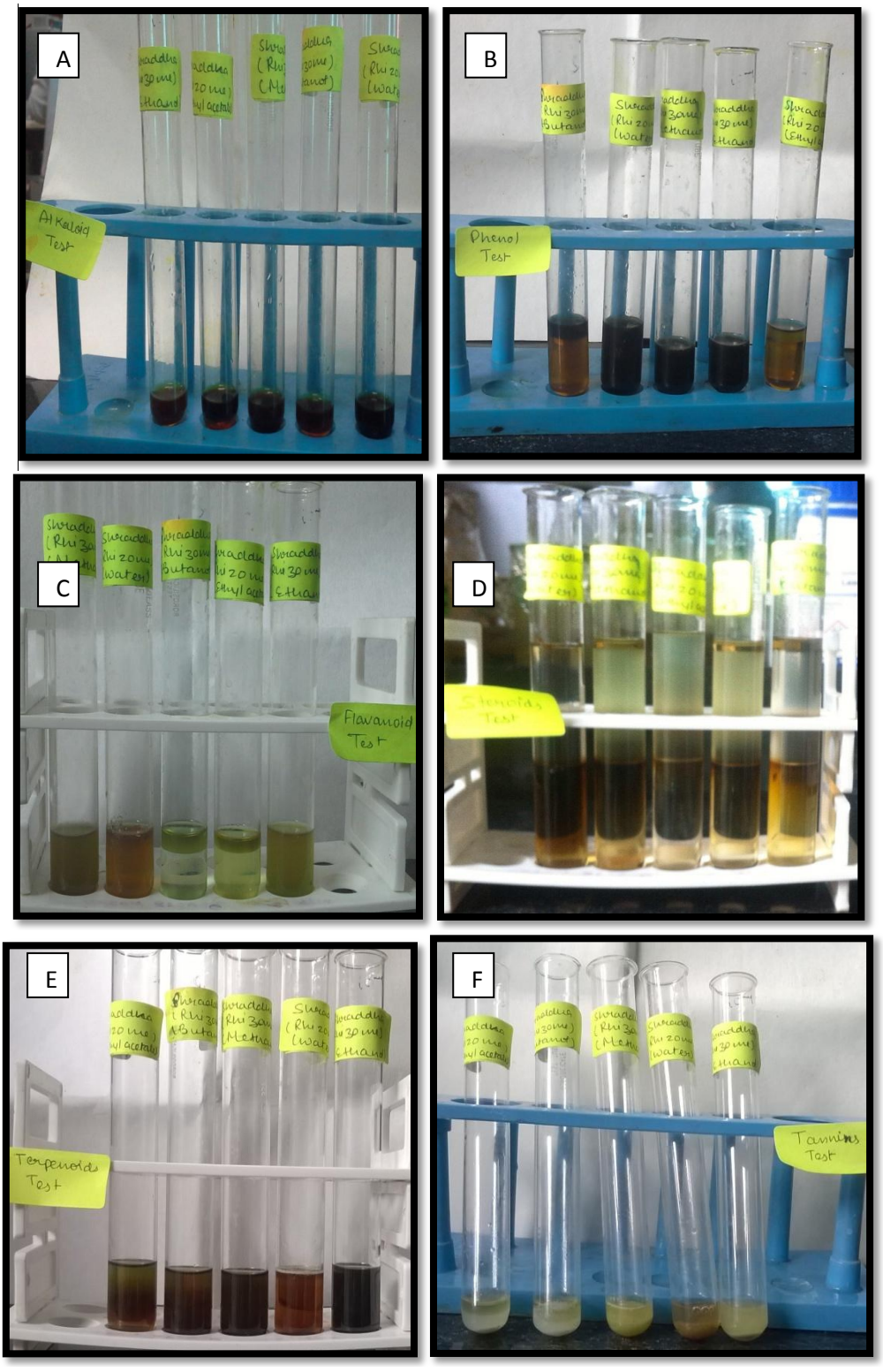
Qualitative phyto-chemical screening of crude extracts of *Alpinia galangal* revealed that alkaloids were present in ethyl acetate and ethanol extracts of both leaf and rhizomes, B extract of rhizome, M extract of leaf and were absent in Aq extract of both, M extract of rhizome and B extract of leaf. Phenols were present in E, M and B extracts of both parts, Aq extract of rhizome, EA extract of leaf and were absent in Aq extract of leaf. Glycosides were present in most of the extracts of both the parts. Tannins, terpenoids, steroids, and flavanoids were present in different extracts of both the parts of plant. But cardiac glycoside was absent in all the extracts leaf of *Alpinia galanga as* demonstrated in Table 2 and 3.

Table 2: Qualitative analysis of various phytochemicals in rhizome of A.galanga

Sr.no	Phytochemicals	Intensity & Inference				
		EA	**B**	**Eth**	**M**	**W**
1	Alkaloids	+	+	+	_	_
2	Phenols	_	+	_	+	+
3	Flavanoids	+	+	_	_	+
4	Steroids	+	_	+	+	+
5	Terpenoids	+	+	_	_	_
6	Tannins	_	_	+	+	+
7	Glycosides	_	+	+	_	+
8	Cardiac glycoside	+	+	_	+	_

++= present in large amount
+=present in small amout
- = absent

EA: Ethyl acetate **B:** Butanol
Eth: Ethanol **M:** Methanol
W: Water


A
Alkaloid Test
B
Phenol Test
C
Flavanoid Test
D
Steroids Test
E
Terpenoids Test
F
Tannins Test

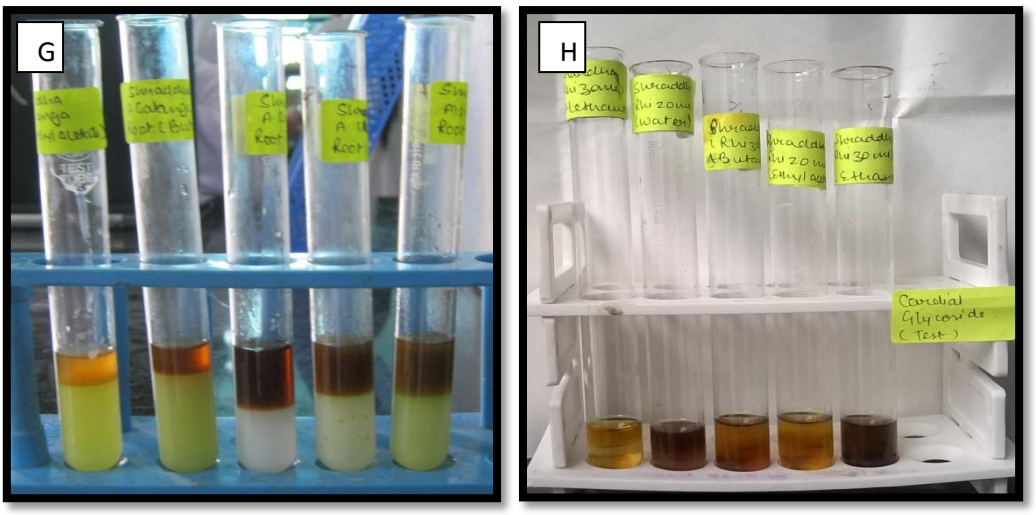


Fig.7: Showing the results for different phytochemical test for rhizome a) Alkaloids b) Phenols c) Flavonoids d) Steroids e) Terpenoids f) Tannins g) Glycosides h) Cardiac glycosides

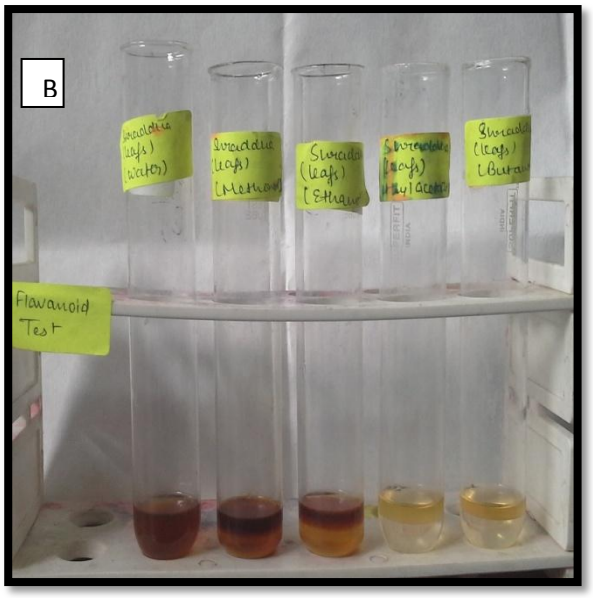
Table 3: Qualitative analysis of various phytochemicals in leafs of A.galanga

Sr.no	Phytochemicals	Intensity & Inference				
		EA	B	Eth	M	W
1	Alkaloids	+	_	+	+	_
2	Phenols	+	+	+	+	_
3	Flavanoids	+	+	+	_	+
4	Steroids	_	+	+	_	_
5	Terpenoids	+	_	_	_	_
6	Tannins	_	_	+	+	_

7	Glycosides	+	+	+	+	+
8	Cardial glycoside	_	_	_	_	_

++= present in large amount
+=present in small amout
- = absent

EA: Ethyl acetate
Eth: Ethanol
W: Water
B: Butanol
M: Methanol



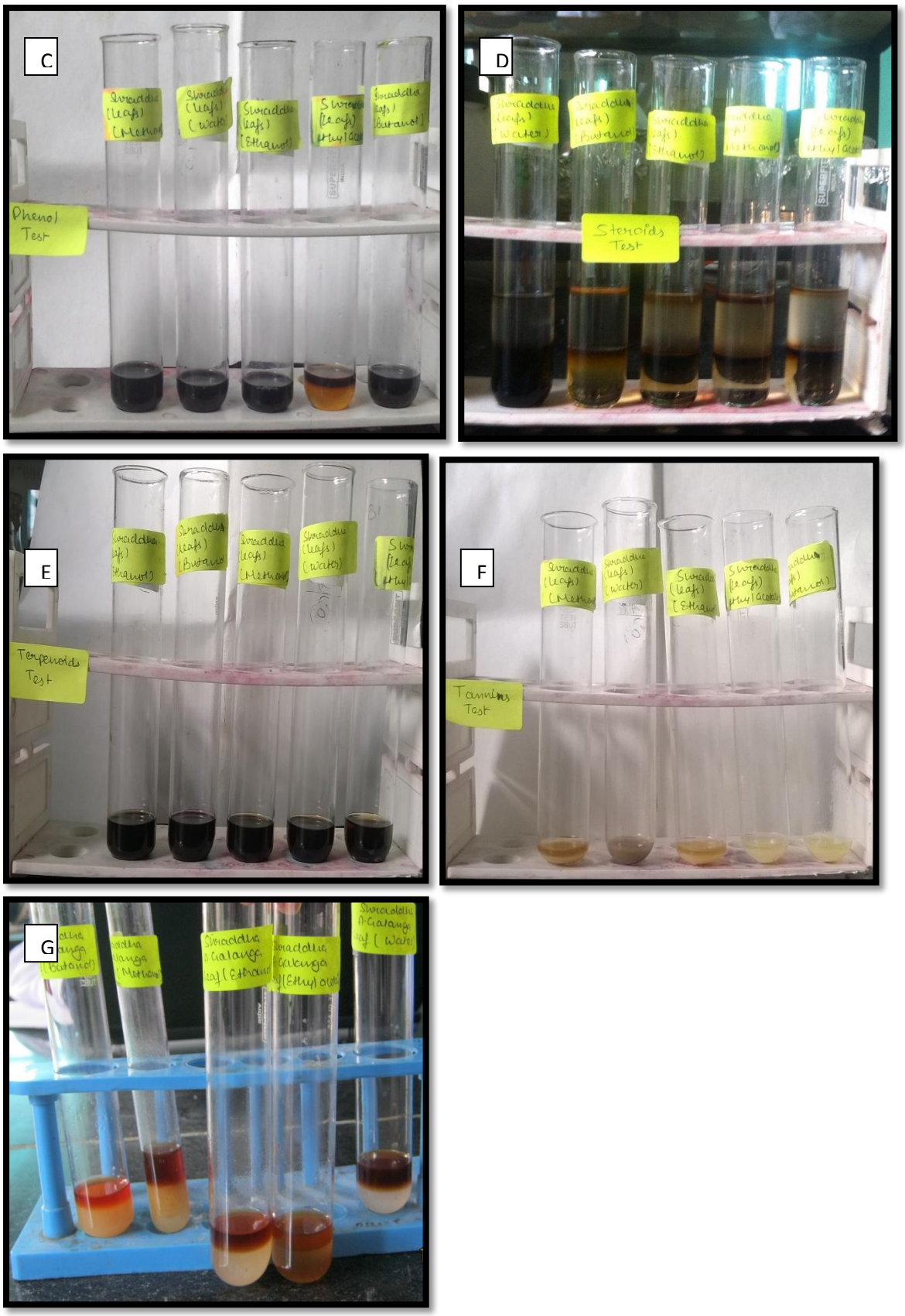


Fig.8: Showing the results of different phytochemical tests for leafs a) Alkaloid b) Phenol c) Flavonoid d) Steroids e) Terpenoids f) Tannins g) Glycoside

6.3 Total flavonoid content

The total flavonoid contents of different solvent extracts of leafs and rhizome parts of *A.galanga* are presented in Table 4,5 and Fig.9. The highest content was obtained in E rhizome(79±0.74) followed by M and EA extracts of rhizome. Lowest content was obtained in B extract (3±0.16) of leafs. Extracts of rhizomes were found to contain higher content than leafs of alpinia galangal. The order of total flavonoid content were E rhizome > M rhizome> EA rhizome > Aq rhizome > Aq leaf> B rhizome> EA leaf> M leaf> E leaf> B leaf.

Table 4: Total flavonoid content for different extracts of rhizome

Rhizome extract	Total Flavonoid content QE(mg/g)
Ethyl acetate	42±0.81
Butanol	14.3±0.47
Ethanol	78±0.81
Methanol	51.6±0.47
Water	34.3±0.47

Values are represented as Mean ± SD, All experiments were repeated thrice

Table 5: Total flavonoid content for different extracts of leaf

Leaf extract	Total Flavonoid content QE(mg/g)
Ethyl acetate	11±0.81
Butanol	2.6±0.47
Ethanol	7.3±0.47
Methanol	10±0.81
Water	17.6±0.47

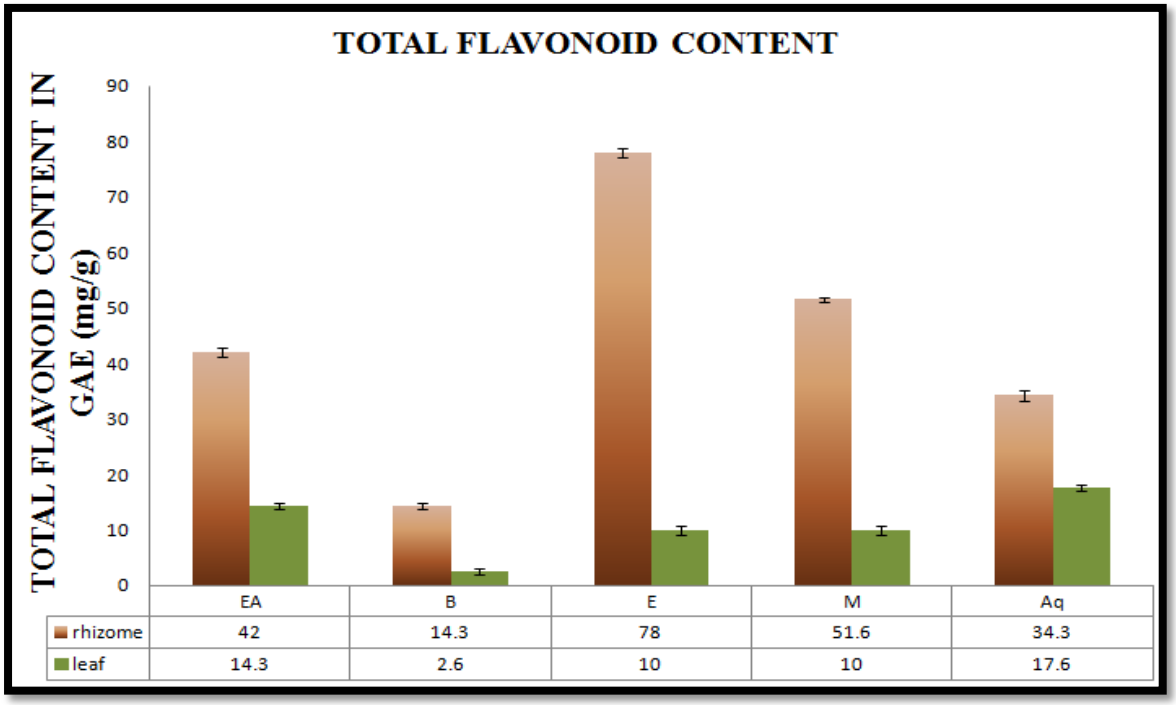


Fig.9: Total flavonoid content in quercetin equivalence (mg/g)

EA: Ethyl acetate **B:** Butanol
Eth: Ethanol **M:** Methanol
W: Water

6.4 Total phenolic content

The highest phenolic content was obtained in EA extract (75±1.08) of rhizome followed by M and E of it. Rhizomes of *galangal* had higher content of phenolic compound than leafs. The B extract of leaf gave lowest level of phenolics(1±0.24) as illustrated in Table 6,7 and Fig.10. The decreasing order of total phenolic content are EA rhizome> M rhizome> E rhizome> M leafs> Aq rhizome> EA leafs> B leaf and rhizome> E leaf> B leaf.

Table 6: Total phenolic content for different extracts of rhizome

Rhizome extract	Total Phenolic content GAE(mg/g)
Ethyl acetate	74.6±0.47
Butanol	17±2.16
Ethanol	39±0.81
Methanol	53±2.16
Water	27.6±0.47

Values are represented as Mean ± SD, All experiments were repeated thrice

Table 7: Total phenolic content for different extract of leaf

Leaf extract	Total Phenolic content GAE(mg/g)
Ethyl acetate	22±0.81
Butanol	1.3±0.47
Ethanol	8.3±1.24
Methanol	35±1.29
Water	15±0.81

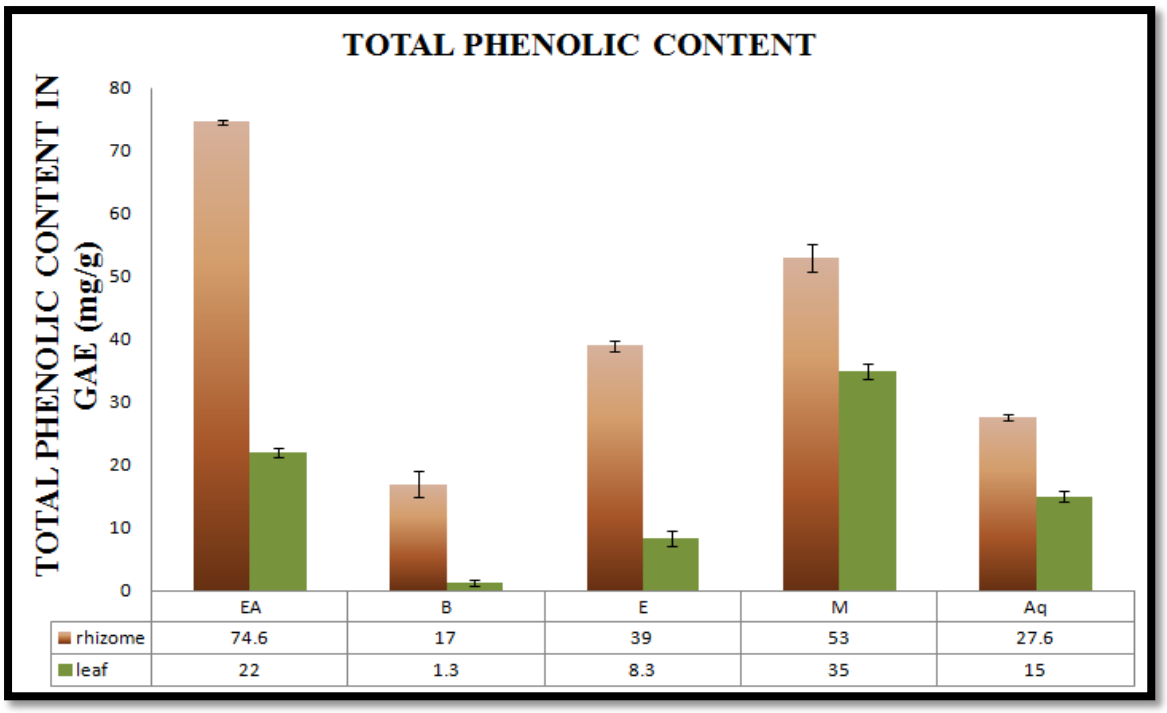


Fig.10: Total phenolic content in gallic acid equivalence (mg/g)

EA: Ethyl acetate
B: Butanol
Eth: Ethanol
M: Methanol
W: Water

6.5 DPPH radical scavenging activity

The amount of DPPH radical scavenging at different concentration of different extracts of leaf and rhizome of *A.galanga* were expressed by % inhibition. Significant differences in scavenging activity were observed as illustrated in Table 8,9 and Fig.11. The highest inhibition of 83% and 60% was shown by M extract of rhizome and Aq extract of leaf, respectively w.r.t. control (ascorbic acid) that is showing 90% at highest concentration. The highest scavenging activity was seen in rhizome M extract (83.2%), followed by rhizome EA (81.33%), rhizome B (79.09%), rhizome E (69.37%), rhizome Aq (68.89%), leaf Aq (60.18%), leaf B (55.92%), leaf EA (55.35%), leaf E (55.25%), leaf M (51.54%) extracts. Aqueous for both was not showing any inhibition. Lower absorbance values show higher free radical scavenging activity.

Table 8: % of DPPH radial scavenging activity for different extracts of rhizome

Extracts	% Scavenging activity			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	34.25 ±0.54	52.04±2.02	61.93±0.96	81.33±1.00
Butanol	37.67±1.10	50.07±0.7	60.17±0.43	79.09±1.14
Ethanol	29.24±1.10	39±1.27	50.33±0.67	69.37±0.55
Methanol	39.31±0.36	53.5±0.4	62±0.13	83.2±0.27
Water	38.3±0.81	41.01±0.12	52.35±0.36	68.89±0.13
Ascorbic acid	37.76±0.37	59.66±0.42	75.6±0.43	90±0.40

Values are represented as Mean ± SD, All experiments were repeated thrice. Ascorbic acid was used as standard.

Table 9: % of DPPH radial scavenging activity for different extracts of leaf

Extracts	% Scavenging activity			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	19.7 ±0.45	24.7±0.46	38.97±0.51	55.35±0.30
Butanol	13.31±0.36	22.08±0.71	38.06±0.62	55.92±0.58
Ethanol	21.66±0.13	30.86±0.23	38.01±0.55	55.25±0.59
Methanol	21.44±0.34	29.91±0.66	39.3±0.81	51.54±0.36
Water	20.01±0.7	25.72±0.12	40.8±0.38	60.18±0.35
Ascorbic acid	37.76±0.37	59.66±0.42	75.6±0.43	90±0.40

Values are represented as Mean ± SD, All experiments were repeated thrice. Ascorbic acid was used as standard.

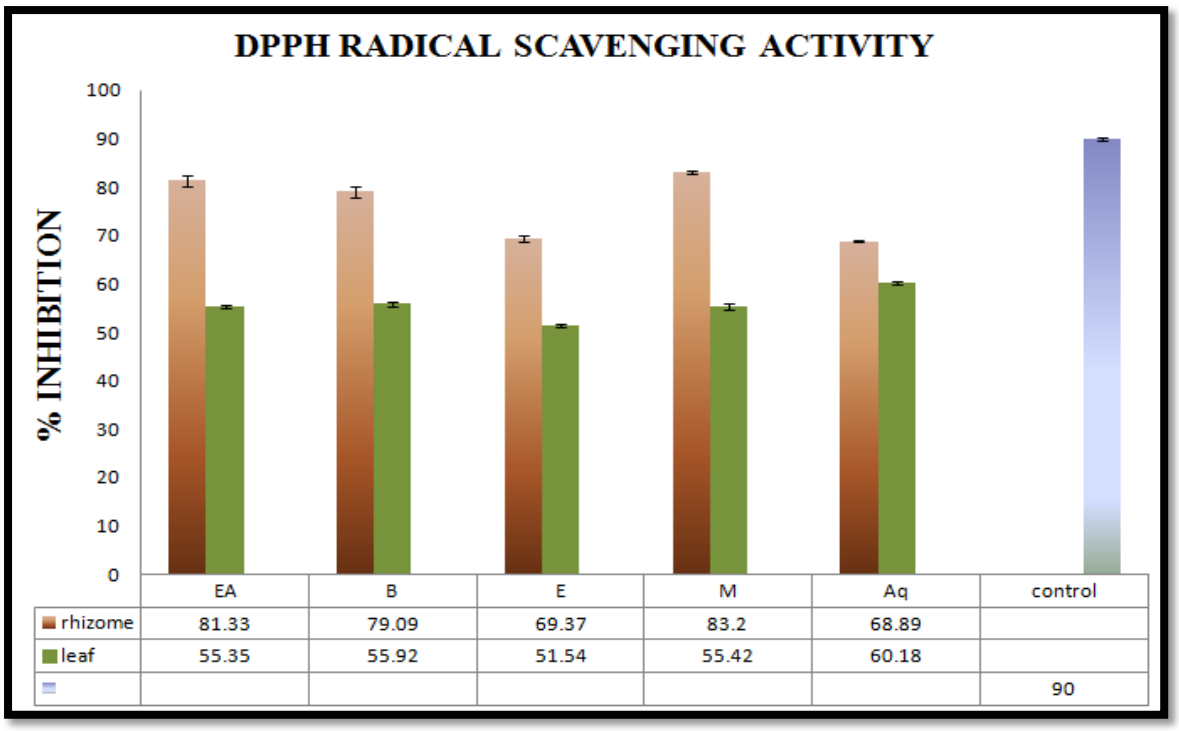


Fig.11: DPPH radical scavenging activity (%)

6.6 Metal ion chelating activity

The chelating effect on the ferrous ions by the various solvent extracts from leaf and rhizome of *A.galanga* had been shown in Table 10, 11 and Fig.12. All the sample extracts exhibited the ability to chelate metal ions. Among the different extracts tested, the B extract from rhizome and B extract from leaf of *A.galanga* showed the highest activity i.e. 84.27% and 62.94% respectively. The decreasing order of % inhibition is as Aq rhizome > M rhizome > E rhizome > EA rhizome > E leaf > M leaf > EA leaf > Aq leaf. Among the different extracts, the Aq extract from rhizome of galangal works best as metal chelator.

Table 10: Showing %inhibition of metal chelating activity for different extracts of rhizome

Extracts	% Inhibition			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	26.54±1.10	40.53±0.88	59.48±1.70	74.10±0.78
Butanol	29.45±0.83	43.23±1.45	58.89±0.68	84.27±1.04
Ethanol	33.7±1.24	46.58±0.68	55.67±0.84	75.27±1.06
Methanol	25.27±0.17	40.64±0.78	60.90±0.82	80.49±1.02
Water	29.24±1.13	43.42±1.11	68.26±0.42	84.2±0.35
Control	28.41±0.63	45.84±0.74	74.33±0.97	92.12±0.64

Values are represented as Mean ± SD, All experiments were repeated thrice.

Table 11: Showing %inhibition of metal chelating activity for different extracts of leaf

Extracts	% Inhibition			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	14.69±0.58	25.20±0.90	39.96±0.35	52.67±0.70
Butanol	15.22±0.30	21.65±1.08	39.29±0.79	62.94±0.50
Ethanol	16.32±0.26	26.28±0.46	38.60±0.81	54.89±1.66
Methanol	19.48±0.62	31.34±0.55	40.63±0.47	53.84±1.44
Water	20.72±1.08	33.57±1.8	41.54±1.53	51.29±0.42
Control	28.41±0.63	45.84±0.74	74.33±0.97	92.12±0.64

Values are represented as Mean ± SD, All experiments were repeated thrice.

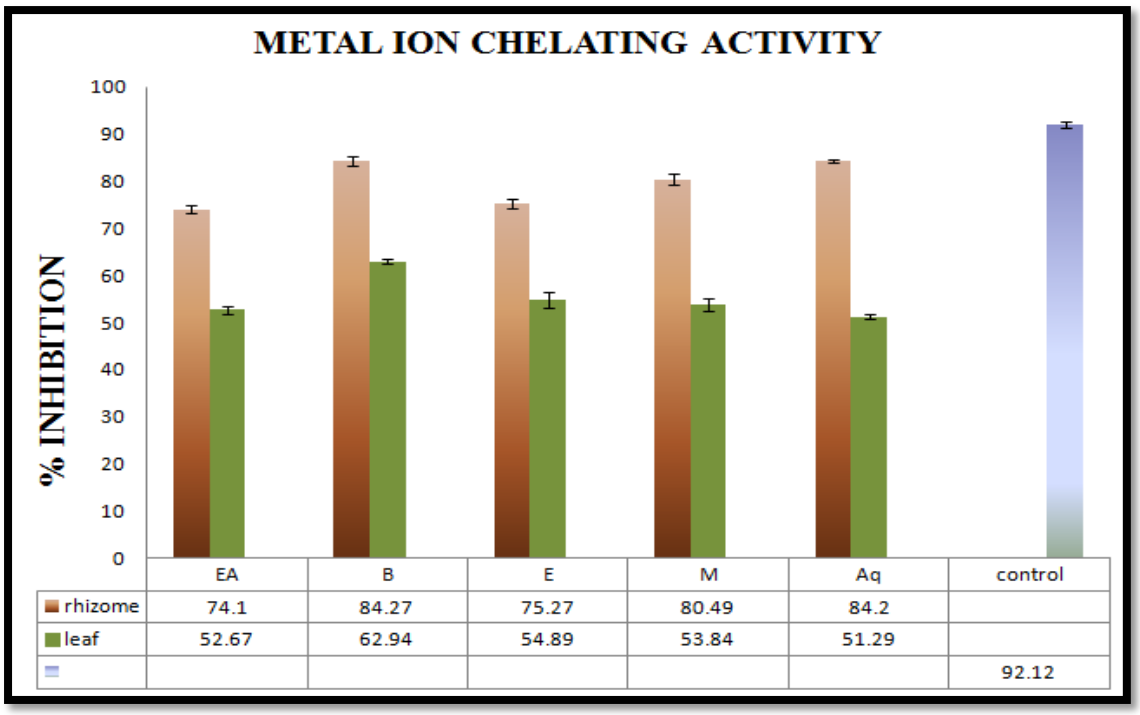


Fig.12: Metal ion chelating activity (%)

6.7 Superoxide anion radical scavenging activity

The superoxide radicals were generated by illuminating a solution containing riboflavin. From table 12, 13 and fig. 13, it was observed that , among all M from rhizome and Aq from leaf showed highest superoxide radical scavenging activity i.e. 83.63% and 64.85% respectively, followed by E rhizome > Aq rhizome > EA rhizome > B rhizome > E leaf > EA leaf > B leaf > M leaf. The rhizome part found to be more effective in scavenging the superoxide radicals.

Table 12: Showing % of superoxide anion radical scavenging activity for different extracts of rhizome

Extracts	% Scavenging activity			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	29.28±0.78	32.72±0.78	57.99±0.32	74.25±0.19
Butanol	31.88±0.28	45.43±0.64	60.74±1.35	73.98±0.22
Ethanol	28.96±1.34	34.75±0.77	51.58±0.77	81.43±0.78
Methanol	26.95±0.31	43.25±1.36	44.55±0.54	83.63±0.47
Water	29.12±1.85	39.52±1.15	60.32±1.27	78.81±0.91
Control	32.15±1.93	51.23±0.94	79.44±0.79	94.50±0.94

Values are represented as Mean ± SD, All experiments were repeated thrice.

Table 13: % of superoxide anion radical scavenging activity for different extracts of leaf

Extracts	% Scavenging activity			
	200 µl/ml	400 µl/ml	600 µl/ml	800 µl/ml
Ethyl acetate	16.30 ±1.07	27.51±0.11	39.15±0.80	56.84±0.29
Butanol	23.41±0.54	26.96±0.15	43.99±0.40	55.35±0.38
Ethanol	20.84±0.28	24.06±0.25	39.72±0.19	59.34±0.35
Methanol	14.55±0.86	23.43±0.56	32.66±0.15	54.08±0.12
Water	23.50±1.01	42.09±1.19	54.10±0.17	64.85±0.67
Control	32.15±1.93	51.23±0.94	79.44±0.79	94.50±0.94

Values are represented as Mean ± SD, All experiments were repeated thrice.

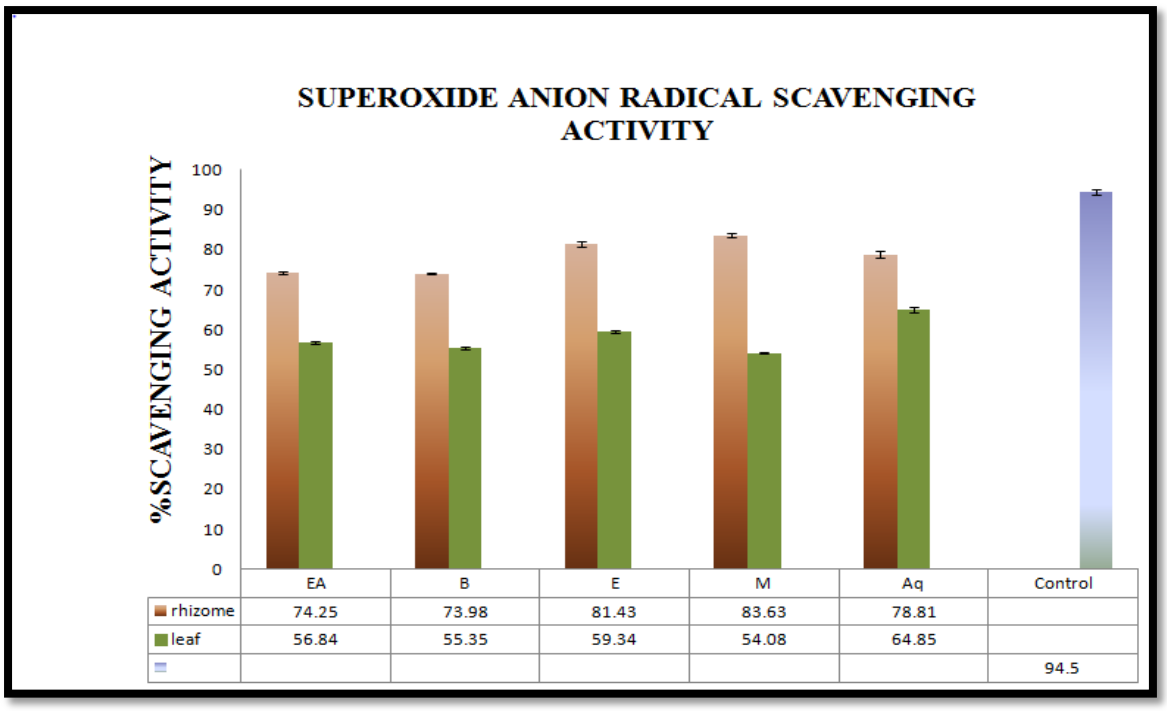


Fig.13: Superoxide anion radical scavenging activity (%)

6.8 Antidiabetic activity

6.8.1 Alpha-amylase inhibitory assay

From the table 14 and fig. 14, M rhizome and E leaf was found to exhibit highest % inhibition among all i.e. 83.39% and 24.34% respectively. The decreasing order of % inhibition of alpha- amylase is E rhizome > EA rhizome > B rhizome > Aq rhizome > EA leaf > B leaf > Aq leaf > M leaf.

Table 14: Showing % inhibition of alpha amylase by different plant extract

Extracts	Parts	
	Rhizome	Leaf
Ethyl acetate	51.25±0.53	22.58±0.76

Butanol	47.74±0.58	16.17±0.39
Ethanol	83.32±0.83	24.34±0.52
Methanol	83.39±0.89	11.72±0.49
Water	44.17±0.74	12.35±0.68
Control	90.64±0.81	90.64±0.81

Values are represented as Mean ± SD, All experiments were repeated thrice.

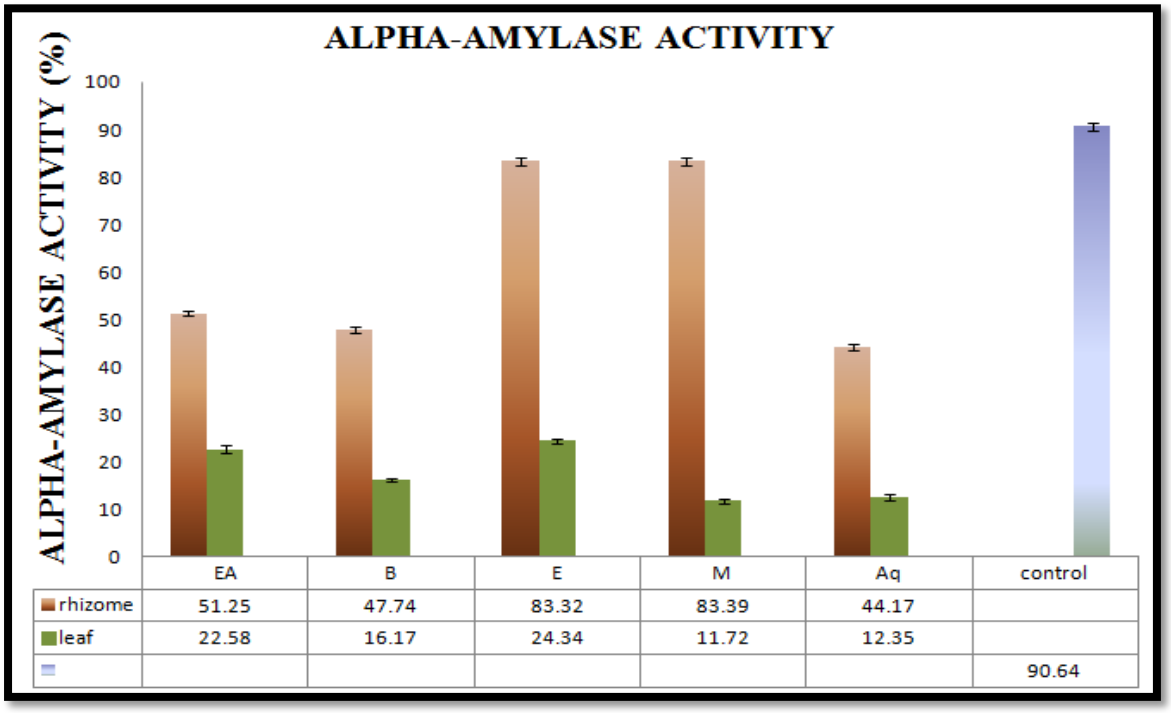


Fig.14: Alpha-amylase activity (%)

9.2 Alpha-glucosidase inhibitory assay

From the table 15 and fig. 15, M rhizome and EA leaf was found to exhibit highest % inhibition among all i.e. 82.45% and 29.39% respectively. The decreasing order of % inhibition of alpha- glucosidase is EA rhizome > E leaf > Aq rhizome > E rhizome > Aq leaf > B rhizome > M leaf > B leaf .

Table 15: Showing % inhibition of alpha glucosidase by different plant extract

Extracts	Parts	
	Rhizome	Leaf
Ethyl acetate	77.18±0.89	29.39±0.73
Butanol	14.52±0.66	9.09±0.39
Ethanol	17.88±0.34	23.07±0.31
Methanol	82.45±0.93	13.07±0.60
water	18.09±0.49	14.80±0.18
Control	88.89±0.37	88.89±0.37

Values are represented as Mean ± SD, All experiments were repeated thrice.

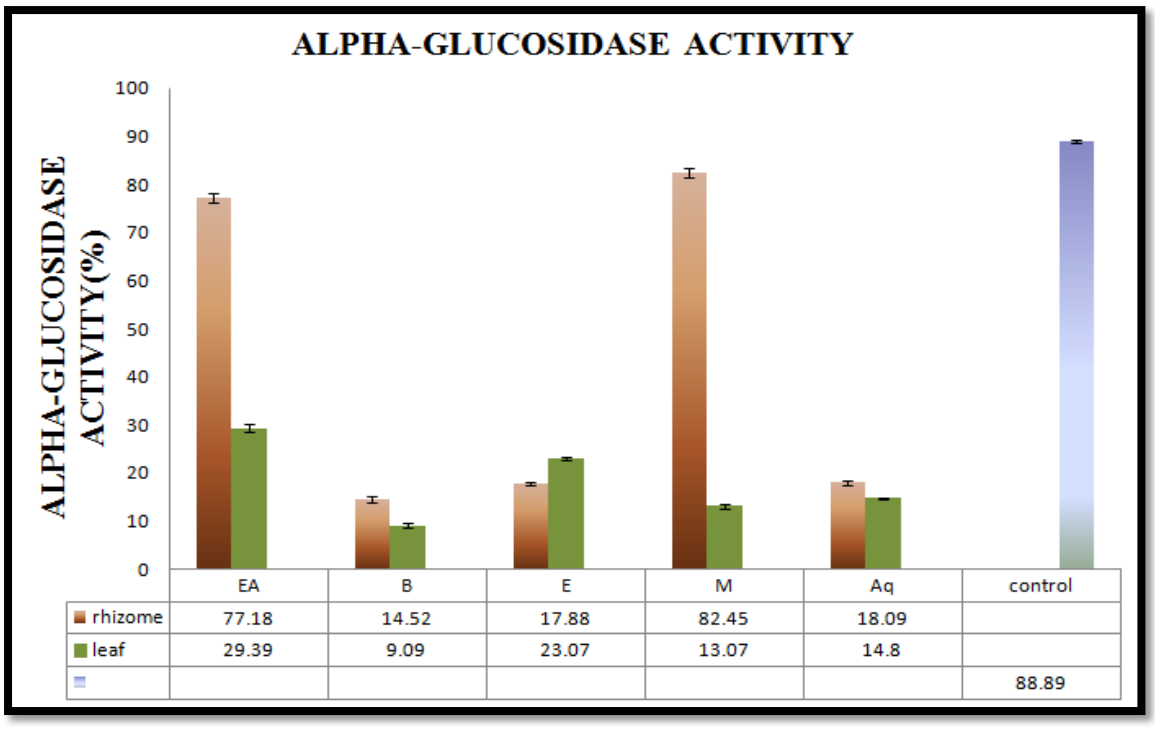


Fig.15: Alpha-glucosidase activity (%)

6.10 Antibacterial activity

The result in table 16, 17 showed that the plant extracts exhibited significant antimicrobial activity. Comparative analysis reveals that B extract from rhizome of plant was found to exhibit highest antibacterial activity against *E.coli* and *B.subtilis*. The highest inhibiton zone diameter (IZD) was recorded on *B.subtilis* i.e. 12.3±0.47 w.r.t. control Gentamycin (18mm). After B rhizome, the highest IZD (7mm) was recorded for B leaf against *B.subtilis* w.r.t. control followed by E leaf (7mm) against *E.coli*. both the strains were found to be resistant against EA extract of both leaf and rhizome. B rhizome showed IZD (4.6mm) against *E.coli* but it was resistant against B leaf. E rhizome showed IZD (5mm) against *E.coli*. But *B.subtilis* was resistant against both E extract of leaf and rhizome. M leaf showed IZD (6mm) against *E.coli* and (5mm) against *B.subtilis*. M rhizome showed IZD (3.7mm) against *B.subtilis* but *E.coli* was resistant to it.

Table 16: Diameter of inhibition zone for *E.coli* in different plant extract

Extracts	Diameter of the inhibition zone (mm)			
	Leaf	Rhizome	Positive control	Negative control
Ethyl acetate	_	_	12.3±0.47	_
Butanol	_	4.6±0.43	10.3±0.47	_
Ethanol	7±0.82	5±0	10±0	_
Methanol	6±0.82	_	12.6±0.47	_
Water	6.3±0.17	_	10±0	_

Values are represented as Mean ± SD, All experiments were repeated thrice. Gentamycin was used as standard and DMSO as negative control in all experiments.

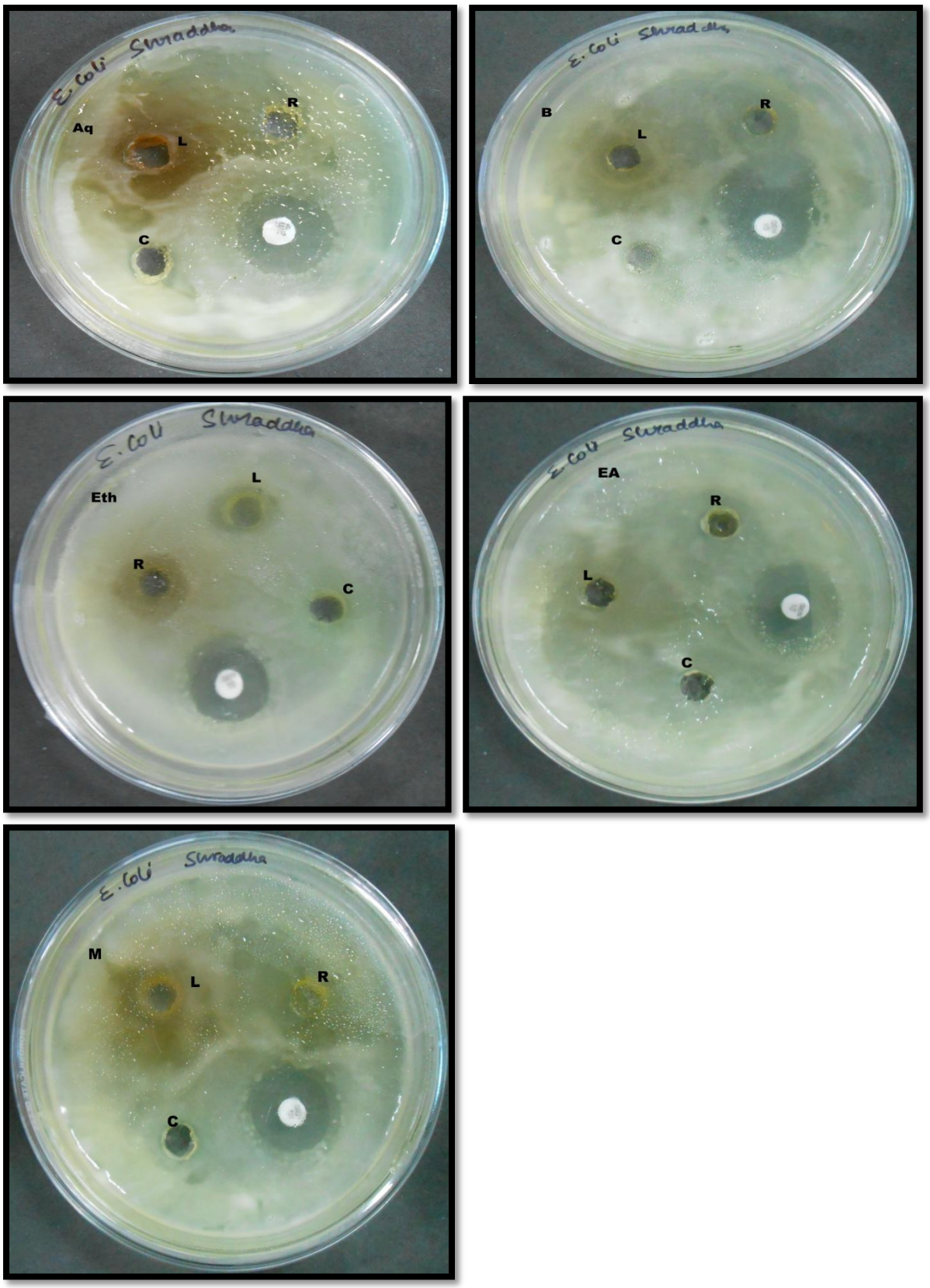


Fig.16: Antibacterial activity of different extracts of leaf and rhizome of *A.galanga* against *E.coli.*

Table 17: Diameter of inhibition zone for *B.subtilis* in different plant extract

Extracts	Diameter of the inhibition zone (mm)			
	Leaf	Rhizome	Positive control	Negative control
Ethyl acetate	_	_	16.6±0.47	_
Butanol	7±0	12.3±0.47	18±0	_
Ethanol	_	_	18.6±0.47	_
Methanol	5±0.82	3.7±0.47	18.3±0.47	_
Water	_	_	18.3±0.47	_

Values are represented as Mean ± SD, All experiments were repeated thrice. Gentamycin was used as standard and DMSO as negative control in all experiments.

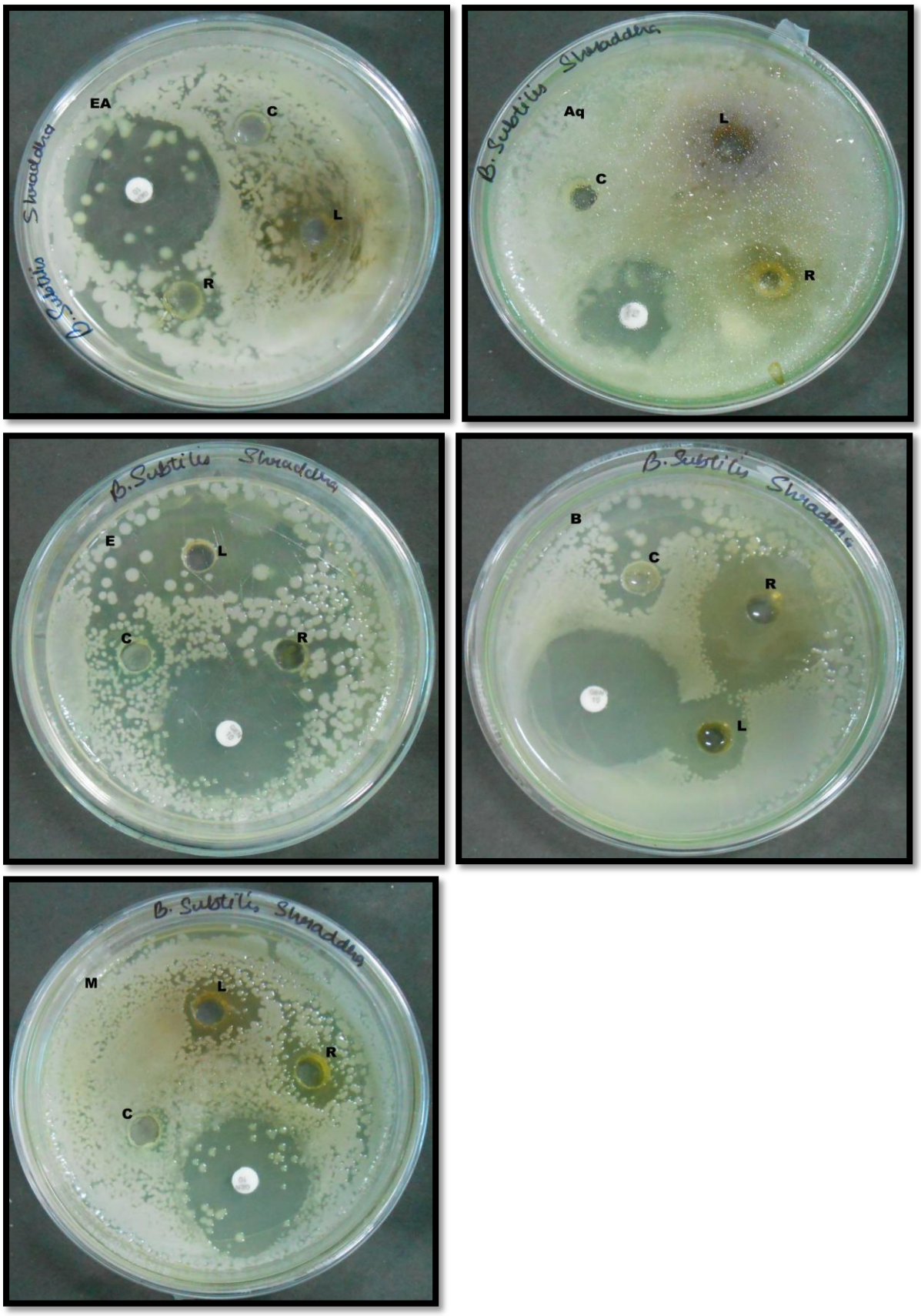


Fig.16: Antibacterial activity of different extracts of leaf and rhizome of *A.galanga* against *E.coli.*

CHAPTER-7

CONCLUSION

Results of present study showed that the extracts from both the parts of *A.galanga* have strong antioxidant activities. Plants are not only a source of food, flavour but are also a source of natural antioxidants. In the present study, the overall results obtained revealed that the level of total phenolic, total flavonoid, DPPH % Inhibition content in both the part of plant extracts were considerable. The highest flavonoid content was present in the E extract of rhizome part and phenolic content was highest in EA extract of leaf part. The highest scavenging activity was seen in M extract of rhizome and Aq extract of leaf. Rhizome of A.galanga contained higher flavonoids as well as phenolics than leaf part, but content of flavonoid was much higher than that of phenolics. Leaf part contained higher phenolics than flavonoids. Rhizome part showed highest antioxidant activity through all the methods. Aq extract of rhizome showed highest %inhibition against metal ions in metal chelating assay and M extract of leaf showed highest scavenging activity against superoxides in superoxide radical scavenging assay. The experiments for antidiabetic revealed that M extract of rhizome exhibit highest activity against both alpha-amylase and alpha-glucosidase. And M extract of leaf and B extract of leaf showed least acivity against both alpha-amylase and alpha-glucosidase, respectively. The comparative analysis showed that, the highest IZD in antibacterial activity was exhibited by E extract of leaf against *E.coli* and by B extract of rhizome against *B.subtilis*. It might be possible for the food industries as well as pharmaceutical industries to formulate drugs using phytochemicals instead of synthetic compounds to reduce health disorders as well as to prevent food deterioration. In conclusion, the results obtained illustrated that these plants are potential sources of antioxidant, antidiabetic and antimicrobial.

Chapter-8

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