IN VITRO PHARMACOLOGICAL INVESTIGATIONS ON DICHLOROMETHANE (DCM) FRACTION OF *SYGYZIUM SAMARANGENSE* LEAF EXTRACT

A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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DECLARATION BY THE CANDIDATE

I, SadiaAfreen Mustafa, hereby declare that this dissertation, entitled **"*In Vitro* Pharmacological Investigations on Dichloromethane (DCM) Fraction of *SygyziumSamarangense* LeafExtract"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me under the guidance of Abdullah-Al-Faysal, Senior Lecturer, Department of Pharmacy, East West University, Dhaka. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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This is to certify that the dissertion, entitled **"*In Vitro* Pharmacological Investigations on Dichloromethane (DCM) Fraction of *SygyziumSamarangense* Leaf Extract"**is a bonafide research work done, under our guidance and supervision by SadiaAfreen Mustafa (ID: 2013-1-70-056), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Dedication

This Research paper is dedicated to

My beloved Parents,

Who are my Biggest Inspiration…

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ABSTRACT

The study was designed for pharmacological investigation of dichloromethane (DCM) fraction of methanol extract of the leaves of *Syzygiumsamarangense*(Family: Myrtaceae). The powdered leaves of *Syzygiumsamarangense*were extracted with methanol and then partitioned with n-hexane, dichloromethane and ethyl acetate consecutively. The DCM fraction remaining was investigated for total flavonoid content, total phenol content,brine shrimp lethality test and antimicrobial test. The fraction contained 21.073 mg AAE/gm of dried extract in total phenolic content assay and 0.581mg quercetin/gm of dried extract in total flavaniod content assay. Screening for cytotoxic properties using brine shrimp lethality bioassay with tamoxifen (LC_{50} value of 13.38µg/ml) as positive control showed that the fraction have considerable cytotoxic potency exhibiting LC_{50} value 2.00 µg/ml. In antimicrobial activity investigation, the DCM fraction showed low to moderate antibacterial and antifungal activity against the tested organisms compared to ciprofloxacin that was used as positive control. The DCM fraction showed strong cytotoxic activity, low antioxidant activity and low to moderate antimicrobial activity. Further investigations are needed for the proper identification and isolation of these bioactive compounds to produce safer drugs for treatment of harmful diseases.

Key words: *Syzygiumsamarangense,* Brine shrimp lethality bio-assay, phenolic content, flavonoid content, antimicrobial activity.

Chapter One
INTRODUCTION

Introduction

1.1. Nature, as a Source of Drugs

Nature, the master of craftsman of molecules created almost an inexhaustible array of molecular entities. It stands as an infinite resource for drug development, novel chemotypes and pharmacophores, and scaffolds for amplification into efficacious drugs for a multitude of disease indications and other valuable bioactive agents. Since time immemorial, natural products have been the backbone of traditional system of healing throughout the globe, and have also been an integral part of history and culture.

Animal Source: The skin of an Ecuadorian poison frog is a source of Epibatidine, which is ten time more potent than morphine.

Plant Source: Withanolides from *Withania somnifera*. They are found to be active in arthritis.

Microbial Source: Penicillin from the filamentous fungus *Penicillium notatum*

Marine Source: Plitidepsin, a depsipeptide was isolated from the Mediterranean Tunicat Aplidium albicans. Plitidepsin is effective in treating various cancers.

Figure 1.1. Different Sources of Drugs

It has been well documented that natural products played critical roles in modern drug development, especially for antibacterial and antitumor agents. Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidates. (Veeresham, 2012)

1.2 Plant Based Drugs and Medicines

Plants are an essential component of the universe. Human beings have used plants as medicine from the very beginning of time. After various observations and experiments medicinal plants were identified as a source of important medicine, therefore, treatment through this medicinal plants, began in the early stages of human civilization .The use of these medicinal plants is increasing in many countries where 35% of drugs contain natural products. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The beneficial medicinal effects of plant materials typically results from combinations of secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body. (Veeresham, 2012)

Morphine

Source: *Papaver somniferum*
Possess Psychotropic and stimulant activity.

Quinine

Source: *Cinchona officinalis*
Antimalerial agent

Digoxin

Source: *Digitalis purpurea*
Used in Conjestive Heart Failure

Nicotine

Source: *Nicotiana Tabacum*
Nicotinic receptor stimulant

Vinka Alkaloids

Source: *Vinka rosea*
Anti-cancer agent

Reserpidine

Source: *Rauwolfia serpentine*
Antihypertensive agent

Figure 1.2. Some Drugs from Plant Source (Siddiqui *et al*., 2014)

Plants have been the part of traditional medicine systems, which have been used for thousands of years in our county. These plant based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization (WHO) that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries. Of these 119 drugs, 74 % were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine. Some examples are given below. (Siddiqui *et al*., 2014)

1.2.1 Anti-inflammatory Agents

Inflammation is known to be one of the important causes responsible for many diseases. Natural products used for inflammation includes Withanolides from *Withania somnifera*. They are found to be active in arthritis and are potent inhibitor of angiogenesis, inflammation and oxidative stress. Inhibition of NFkB and NFkB regulated gene expression is primarily responsible for their anti-arthritis action. Another prominent example is *Salai guggal* (Boswellia serrata) which was investigated at IIM, JAMMU and also show anti-arthritis action. Alkaloid, berberine from *Berberis aristata* also have anti-inflammatory action by inhibition of NFkB, COX2, TNF, IL-I, IL- 6. Another prominent example is Curcumin from Turmeric *Curcuma longa*, reported in 1971 to be an effective anti-inflammatory agent at CDRI LUCKNOW, show broad spectrum activity on inflammation. Another substance is Guggulsterone from *Commiphora mukul* (guggul). Nimbidin from neem (*Azadirachta indica*) and Embel a constituent of Vidang (*Embelia ribes*) also show anti-inflammatory action.

Figure 1.3

Berberine

Source: *Berberis aristata*

Have anti-inflammatory action.

1.2.2 Cardio-vascular Agents

Cardiac glycosides or cardenolides are commonly used. They are steroidal in nature with a lactone group. They inhibit the membrane bond Na-K ATPase pump resulting in depletion of intracellular K and increase in serum K which result in decrease electrical conductivity through a decrease in heart rate and increase cardiac output.

Yellow oleander plant (*Thevetia neriifolia*) have thevitin A, B and peruvoside which are potent cardiac glycoside. *Rauwolfia serpentine* contain reserpine, was first tested in INDIA for anti-hypertensive activity. It inhibits action by inhibiting mono amine oxidase (MAO). The *Terminalia arjuna* bark has been used for treatment of angina. Arjunolic acid is main constituent to exhibit this action.

Figure 1.4

***Terminalia arjuna* Bark**

Used for treatment of angina. Arjunolic acid is main constituent to exhibit this action.

The Coleus spp have also been reported in materia medica for treatment of heart disease. Digoxin obtained from *Digitalis purpurea* is most widely used cardenolides. Another most important example is Quinidine from *Cinchona officinalis* use as antiarrythmic agent.

1.2.3 Anti-diabetic agents

India is a 'Diabetic capital of world' several remedies are used for their treatment. Most common example is Charantin a steroidal saponin have an insulin like activity. *Sylvestre Gymnema* (gurmar) from which gymnemic acid is obtain known to show hypoglycaemic activity. *Momordica charantia* commonly known as Karela have momordicoside which is used for diabetes. Andrographolide a diterpenoid lactone from *Andro graphis* Paniculata has been found to exhibit significant hypoglycaemic activity. *Syzygium jambolanium* have anthocyanins which are responsible for antidiabetic action. Liquiritigenin extracted from *Pterocarpus* marsupium also another important example. *Trigonella foneum*commonly known as fenugreek, shows potent anti-diabetic action.

Figure 1.5

Sylvestre Gymnema

Gymnemic acid is obtain known to show hypoglycaemic activity.

1.2.4 Anti-obesity Agents

There are many natural products that have been used for anti-obesity agent. Tea polyphenolics like 3-o-gallate show a potent lipase inhibitor activity. 3-Methylethergangalin and 5-hydroxy- 7-

(4'hydroxy-3'-methoxyphenyl)-1- phenyl-3- heptanone isolated *from Alpinia officinarum* have shown significant lipase inhibitory action.

Figure 1.6

Alpinia officinarum

Have shown significant lipase inhibitory action.

Garcinia cambogia have hydroxyl citric acid which is used as an antiobestiy agent. Guggulipid, a fraction of *Commiphora mukul* resin and has been developed at Lucknow, and have guggulsterone act as hyperlipidameic agent.

1.2.5 Anti-malarial Agents

A number of medicinal plants have been used traditionally in the treatment of malaria. Several biflavonoids from *Selaginella Bryopteris* which includes amentoflavone, have been investigated for their anti-protozoal activity in vitro against K strain of *Plasmodium falciparum*. Neem which have nimbolides is used as an antimalarial agent. Naphthylisoquinoline alkaloids isolated from leaves of *Anastrocladus heyneanus* particularly anastrocladidine, ancistrocladidine, ancistrocladinium B and ancistrotanzanine have been shown to exhibit significant anti plasmodial activity. Arteether derived from artemisinin, was first isolated from the plant *Artemisia annua* was approved as antimalarial drugs. Quinine from *Cinchona officinalis* is a potent antimalarial agent.

Figure 1.7

Selaginella Bryopteris

Includes amentoflavone have been investigated for their anti-protozoal activity.

1.2.6 Immunomodulators

An immune modulator is defined as a biological or non-biological substance that directly influences a specific immune function or modifies one or more components of immune regulatory network to achieve an indirect effect on a specific immune function. Many plant derived natural products have been found as an immunomodulator. The immuno-suppressant property of 5, 20-(R)-Dihydroxy-6, 7-epoxy-1-oxo-(5)-with a 2, 24-dienolide from *Withania somnifera* and the steroidal alkaloid solasodine from *Solanum nigrum*, are used as immunomodulators. *Picrorrhiza kurroa* was another example, the active constituent is known kutkin and is a mixture of Kutkoside and Picroside.

Figure 1.8

Solanum nigrum

Used as immunomodulators.

1.2.7 Anti-leshmanial Agents

A large number of molecule belonging to various class of natural products have been isolated which include Diospyrin. It has been isolated from *Diospyros spp*. And found to have very potent antileishmanial activity against *Leishmania donavani*. Plumbagin from*Plumbago spp*. is perhaps the most potent agent. Berberine from *Berberis aristata* is another prominent example. Piperine which is found from Piper species used against promastigotis of *L. Donavani* with activity comparable to pentamidine. Amarogentin isolated from *Swertia chirata* has been found to inhibit *L.donovani* topoisomerase I.

Figure 1.9

Swertia chirata

Amarogentin has been found to inhibit *L.donovani*topoisomeraseI.

Besides these compounds, Picroliv a standardized mixture of iridoid glycosides prepared from the root and rhizome extract of *Picrorrhiza Kurroa* shows a significant anti leshmanial activity and used in combination therapy of Kala azar fever with Na stibogluconate. It is reported to enhance the efficacy of the anti leishmanial drug and also to reduce its side effects.

1.2.8 Antiviral Agents

Several natural products have been used as anti-viral drug which include alkaloids, phenolids and terpenoids. Theasinensin a phenolic compound found in Tea (*thea sinensis*) has been shown to exhibit a good antiviral activity. The common phytosterols ursolic acid and oleanolic acid found in many plants also used as anti HIV agent. Gallic acid chebulagic acid and other galloyl glucose

isolated from *Terminalia chebula* have been reported to show a promising HIV integrase inhibitory activity. Termilignan, Thannilignan 7-hydroxy-3, 4-(methylene dioxy) - flavones and anolignan B isolated from fruit rinds of *Terminalia belerica* have been reported as anti HIV agent.

Figure 1.10

Tea (*Thea sinensis*)

Has been shown to exhibit a good antiviral activity.

1.2.9 Antineoplastic Agents

There are few example of natural products which have been used as antineoplastic agent. Arnebin a napthoquinone found in an*Arnebia nobeles* have been found to be active against walker carcinoma in rats. A diterpenoid precalyone isolated from *Roylea calyana* also found useful against lymphoid leukaemia. The other example include Tagitinin F, a germacranolide isolated from *Tithonia tagitiflora* has been also found to be active against lymphocytic leukaemia. Flavoperidol a semi synthetic flavonoid is to be tested in clinical trials. Combretastatins found in species of Combretacea family have reported used in cancer. *Podophyllum emodii* has been used in skin cancers and warts.(Siddiqui *et al*., 2014)

Figure 1.11

Podophyllum emodii

Has been used in skin cancers and warts. (Siddiqui *et al*., 2014)

1.3 Drugs from Plant Origin

Many valuable drugs are made from plants. Here are a few plants and plant ingredients used in many conventional drugs.

Table 1.1. Drugs from different plant origin. (Taylor, 2000)

Drug/Chemical	Action/Clinical Use	Plant Source
Acetyldigoxin	Cardiotonic	*Digitalis lanata*
Adoniside	Cardiotonic	*Adonis vernalis*
Aescin	Anti-inflammatory	*Aesculus hippocastanum*
Aesculetin	Anti-dysentery	*Frazinus rhychophylla*
Agrimophol	Anthelmintic	*Agrimonia supatoria*
Ajmalicine	Circulatory Disorders	*Rauvolfia sepentina*
Allantoin	Vulnerary	*Several plants*
Allyl isothiocyanate	Rubefacient	*Brassica nigra*

Anabesine	Skeletal muscle relaxant	*Anabasis sphylla*
Andrographolide	Baccillary dysentery	*Andrographis paniculata*
Anisodamine	Anticholinergic	*Anisodus tanguticus*
Anisodine	Anticholinergic	*Anisodus tanguticus*
Arecoline	Anthelmintic	*Areca catechu*
Asiaticoside	Vulnerary	*Centella asiatica*
Atropine	Anticholinergic	*Atropa belladonna*
Benzyl benzoate	Scabicide	*Several plants*
Berberine	Bacillary dysentery	*Berberis vulgaris*
Bergenin	Antitussive	*Ardisia japonica*
Betulinic acid	Anticancerous	*Betula alba*
Borneol	Antipyretic, analgesic, antiinflammatory	*Several plants*
Bromelain	Anti-inflammatory, proteolytic	*Ananas comosus*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubefacient	*Cinnamomum camphora*
Camptothecin	Anticancerous	*Camptotheca acuminata*
(+)-Catechin	Haemostatic	*Potentilla fragarioides*
Chymopapain	Proteolytic, mucolytic	*Carica papaya*
Cissampeline	Skeletal muscle relaxant	*Cissampelos pareira*

Cocaine	Local anaesthetic	*Erythroxylum coca*
Codeine	Analgesic, antitussive	*Papaver somniferum*
Colchiceine amide	Antitumor agent	*Colchicum autumnale*
Colchicine	Antitumor agent, anti-gout	*Colchicum autumnale*
Convallatoxin	Cardiotonic	*Convallaria majalis*
Curcumin	Choleretic	*Curcuma longa*
Cynarin	Choleretic	*Cynara scolymus*
Danthron	Laxative	*Cassia species*
Demecolcine	Antitumor agent	*Colchicum autumnale*
Deserpidine	Antihypertensive, tranquillizer	*Rauvolfia canescens*

1.4 Drug Development Process

Bringing one new drug to the public typically costs a pharmaceutical or biotechnology company on average more than $1 billion and takes an average of 10 to 15 years. PPD's comprehensive portfolio of services, spanning preclinical through post-approval, features integrated laboratory, Phase I-IV and consulting services to provide end-to-end solutions. (Balandri, Kinghorn and Fransworth, 1993)

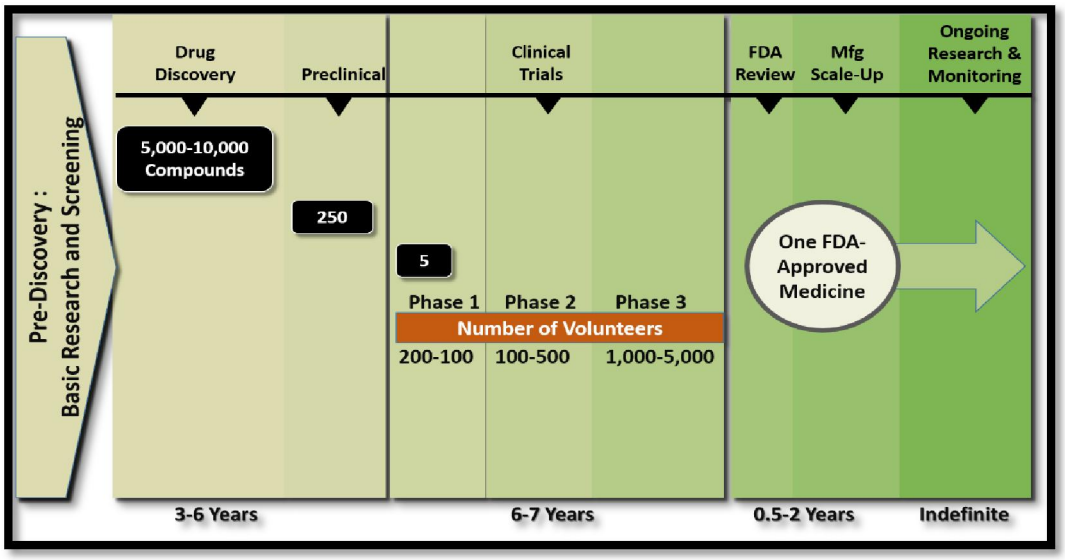


Figure 1.12. Drug Development process

1.4.1 Drug Discovery and Development

New drugs begin in the laboratory with scientists, including chemists and pharmacologists, who identify cellular and genetic factors that play a role in specific diseases. They search for chemical and biological substances that target these biological markers and are likely to have drug-like effects. Out of every 5,000 new compounds identified during the discovery process, approximately five are considered safe for testing in human volunteers after preclinical evaluations. After three to six years of further clinical testing in patients, only one of these compounds on average is ultimately approved as a marketed drug for treatment. The following sequence of research activities begins the process that results in development of new medicines.

❖ Target Identification

Drugs usually act on either cellular or genetic chemicals in the body, known as targets, which are believed to be associated with disease. Scientists use a variety of techniques to identify and isolate individual targets to learn more about their functions and how they influence disease.

Compounds are then identified that have various interactions with the drug targets that might be helpful in treatment of a specific disease.

❖ Target Prioritization and Validation

To select targets most likely to be useful in the development of new treatments for disease, researchers analyze and compare each drug target to others based on their association with a specific disease and their ability to regulate biological and chemical compounds in the body. Tests are conducted to confirm that interactions with the drug target are associated with a desired change in the behavior of diseased cells. Research scientists can then identify compounds that have an effect on the target selected.

❖ Lead Identification

A lead compound or substance is one that is believed to have potential to treat disease. Laboratory scientists can compare known substances with new compounds to determine their likelihood of success. Leads are sometimes developed as collections, or libraries, of individual molecules that possess properties needed in a new drug. Testing is then done on each of these molecules to confirm its effect on the drug target.

❖ Lead Optimization

Lead optimization compares the properties of various lead compounds and provides information to help biopharmaceutical companies select the compound or compounds with the greatest potential to be developed into safe and effective medicines. Often during this same stage of development, lead prioritization studies are conducted in living organisms (*in vivo*) and in cells in the test tube (*in vitro*) to compare various lead compounds and how they are metabolized and affect the body. (Balandri, Kinghorn and Fransworth, 1993)

1.4.2 Requirementsbefore an investigational drugs can be tested in humans

In the preclinical stage of drug development, an investigational drug must be tested extensively in the laboratory to ensure it will be safe to administer to humans. Testing at this stage can take from one to five years and must provide information about the pharmaceutical composition of the drug, its safety, how the drug will be formulated and manufactured, and how it will be administered to the first human subjects.

❖ Preclinical Technology

During the preclinical development of a drug, laboratory tests document the effect of the investigational drug in living organisms (*in vivo*) and in cells in the test tube (*in vitro*).

❖ Chemistry Manufacturing and Controls (CMC)/Pharmaceutics

The results of preclinical testing are used by experts in pharmaceutical methods to determine how to best formulate the drug for its intended clinical use. For example, a drug that is intended to act on the sinuses may be formulated as a time-release capsule or as a nasal spray. Regulatory agencies require testing that documents the characteristics such as chemical composition, purity, quality and potency of the drug's active ingredient and of the formulated drug.

❖ Pharmacology/Toxicology

Pharmacological testing determines effects of the candidate drug on the body. Toxicology studies are conducted to identify potential risks to humans.

Results of all testing must be provided to the Food and Drug Administration (FDA) or other appropriate regulatory agencies to obtain permission to begin clinical testing in humans. Regulatory agencies review the specific tests and documentation required to proceed to the next stage of development. (Balandri, Kinghorn and Fransworth, 1993)

1.4.3 Process of Investigational drugs being tested in humans

Testing of an investigational new drug begins with submission of information about the drug and application for permission to begin administration to healthy volunteers or patients.

- **Investigational New Drug (IND) / Clinical Trial Exception (CTX) / Clinical Trial Authorization (CTA) Applications**

INDs (in the U.S.), CTXs (in the U.K.) and CTAs (in Australia) are examples of requests submitted to appropriate regulatory authorities for permission to conduct investigational research. This research can include testing of a new dosage form or new use of a drug already approved to be marketed.

In addition to obtaining permission from appropriate regulatory authorities, an institutional or independent review board (IRB) or ethical advisory board must approve the protocol for testing, as well as the informed consent documents that volunteers sign prior to participating in a clinical study. An IRB is an independent committee of physicians, community advocates and others that ensures a clinical trial is ethical and the rights of study participants are protected.

Clinical testing is usually described as consisting of Phase I, Phase II and Phase III clinical studies. In each successive phase, increasing numbers of patients are tested.

Phase I Clinical Studies

Phase I studies are designed to verify safety and tolerability of the candidate drug in humans and typically take six to nine months. These are the first studies conducted in humans. A small number of subjects, usually from 20 to 100 healthy volunteers, take the investigational drug for short periods of time. Testing includes observation and careful documentation of how the drug acts in the body -- how it is absorbed, distributed, metabolized and excreted.

Phase II Clinical Studies

Phase II studies are designed to determine effectiveness and further study the safety of the candidate drug in humans. Depending upon the type of investigational drug and the condition it

treats, this phase of development generally takes from six months to three years. Testing is conducted with up to several hundred patients suffering from the condition the investigational drug is designed to treat. This testing determines safety and effectiveness of the drug in treating the condition and establishes the minimum and maximum effective dose. Most Phase II clinical trials are randomized, or randomly divided into groups, one of which receives the investigational drug, one of which gets a placebo containing no medication and sometimes a third group that receives a current standard treatment to which the new investigational drug will be compared. In addition, most Phase II studies are double-blinded, meaning that neither patients nor researchers evaluating the compound know who is receiving the investigational drug or placebo.

Phase III Clinical Studies

Phase III studies provide expanded testing of effectiveness and safety of an investigational drug, usually in randomized and blinded clinical trials. Depending on the type of drug candidate and the condition it treats, this phase usually requires one to four years of testing. In Phase III, safety and efficacy testing is conducted with several hundred to thousands of volunteer patients suffering from the condition the investigational drug treats.

- **New Drug Application (NDA)/Marketing Authorization Application (MAA)**

NDAs (in the U.S.) and MAAs (in the U.K.) are examples of applications to market a new drug. Such applications document safety and efficacy of the investigational drug and contain all the information collected during the drug development process. At the conclusion of successful preclinical and clinical testing, this series of documents is submitted to the FDA in the U.S. or to the applicable regulatory authorities in other countries. The application must present substantial evidence that the drug will have the effect it is represented to have when people use it or under the conditions for which it is prescribed, recommended or suggested in the labeling. Obtaining approval to market a new drug frequently takes six months to two years. (Balandri, Kinghorn and Fransworth, 1993)

1.5 The Role of Plant-Derived Natural Products in Modern Medicine

The commercial value of drug products still derived directly from higher plants is considerable and should not be underestimated. For example, in 1980 American consumers paid about $8 billion for prescription drugs derived solely from higher plant sources. From 1959 to 1980, drugs derived from higher plants represented a constant 25% of all new and refilled prescriptions dispensed from community pharmacies in the United States. Plant-derived drugs thus represent stable markets upon which both physicians and patients rely. In addition, worldwide markets in plant-derived drugs are difficult to estimate, but undoubtedly amount to many additional billions of dollars.

Plants continue to be important sources of new drugs, as evidenced by the recent approvals in the United States of several new plant-derived drugs, and semi-synthetic and synthetic drugs based on plant secondary compounds. For example, taxol, an anticancer. Taxane diterpenoid is derived from the relatively scarce Pacific or western yew tree, Taxus brevifolia Nutt has been approved in the for the treatment of refractory ovarian cancer. Etoposide is a relatively new semisynthetic antineoplastic agent based on podophyllotoxin, a constituent of the mayapple, *Podophyllum peltatum L*, which is useful in the chemotherapeutic treatment of refractory testicular carcinomas, small cell lung carcinomas, nonlymphocytic leukemias, and non-Hodgkin's lymphomas. Atracurium besylate is a relatively new synthetic skeletal muscle relaxant which is structurally and pharmacologically related to the curare alkaloids.

In addition, synthetic A9- tetrahydrocannabinol (originally derived from the marijuana plant, *Cannabis sativa L.*) and some of its synthetic analogs (e.g., nabilone) have recently been approved for the treatment of the nausea associated with cancer chemotherapy. Cannabinoids are also being developed for use in glaucoma and in neurological disorders (e.g., epilepsy and dystonia), and as antihypertensives (cardiovascular agents), antiasthmatics (bronchodilators), and potent analgesics.

Cannabinoids

Taxol

Etoposide

Figure 1.13. Plant derived products

Plant-derived drugs which are currently undergoing development and testing include the Chinese drug artemisinin (qinghaosu) and several of its derivatives, which are newly discovered rapidly acting antimalarial agents derived from *Artemisia annua L*, and forskolin, a naturally occurring labdane diterpene with antihypertensive, positive inotropic, and adenyl cyclase-activating properties. Forskolin is derived from the root of *Coleus forskohlii* Briq, a plant used in East Indian folk medicine and cited in ancient Hindu and Ayurvedic texts.

In addition to the compounds mentioned above, other bioactive plant secondary metabolites are being investigated for their potential utility. For example, the medicinally active organosulfur compounds of garlic and onions are currently being investigated and evaluated as potentially useful cardiovascular agents, and ellagic acid, p-carotene, and vitamin E (tocopherols) are being tested and evaluated for their possible utility as prototype anti-mutagenic and cancer-preventing agents.

In the future, advances in our understanding of immunology and related areas should permit the development of new selective and sensitive bioassays to guide the isolation of bioactive natural products. These continuing developments should provide the means for identifying new plant-derived antiviral, antitumor, immunostimulating, and adaptogenic agents (adaptogens reportedly increase stress tolerance). Potential adaptogenic crude drugs worthy of detailed investigation include American ginseng,Asiatic ginseng, and eleuthero or Siberian ginseng. (Balandri, Kinghorn and Fransworth, 1993)

1.5.1 The Role of Plant-Derived Natural Products as Lead Compounds for the Design, Synthesis, and Development of Novel Drug Compounds

Many other bioactive plant compounds have proven useful as "leads" or model compounds for drug syntheses or semisyntheses. Natural compounds of pharmaceutical importance that were once obtained from higher plant sources, but which are now produced commercially largely by synthesis, include caffeine, theophylline, theobromine, ephedrine, pseudoephedrine, emetine, papaverine, L-dopa, salicylic acid, and A9-tetrahydrocannabinol. In addition, p-carotene, a plant primary metabolite which may be useful in the prevention and treatment of certain cancers, is currently produced synthetically on a commercial scale.

However, despite these numerous examples of economically synthesizable natural products, it is frequently forgotten that plant secondary compounds can, and often do, serve additionally as chemical models or templates for the design and total synthesis of new drug entities. For example, the belladonna alkaloids (e.g., atropine), physostigmine, quinine, cocaine, gramine, the opiates (codeine and morphine), papaverine, and salicylic acid have served as models for the design and synthesis of anticholinergics, anticholinesterases, antimalarial drugs, benzocaine, procaine, lidocaine (Xylocaine) and other local anesthetics, the analgesics pentazocine (Talwin), propoxyphene (Darvon), methadone, and meperidine (Demerol), verapamil (a near-anagram of the words Papaver and papaverine), and aspirin (acetylsalicylic acid), respectively

Similarly, the study of synthetic analogs of khellin, a furanochromone derived from the fruits of *Ammi visnaga*, formerly marketed as a bronchodilator and coronary medication, led to the preparation and development of sodium chromoglycate, also known as chromolyn sodium. Chromolyn is now a major drug used as a bronchodilator and for its antiallergenic properties.

Related synthetic studies based on the benzofuran moiety eventually led to the development of amiodarone, which was originally introduced in Europe as a coronary vasodilator for angina, but which was subsequently found to have a more useful application in the treatment of a specific type of arrhythmia, the Wolff Parkinson-White syndrome, and for arrhythmias resistant to other drugs. It was introduced as a drug in the U.K. in 1980 and most recently in the United States. In still another example, the guanidine-type alkaloid, galegine, was found to be the active principle of goat's rue (*Galega officinalis L.*), and was used clinically for the treatment of diabetes.

It had been known for some time previous to this discovery that guanidine itself had antidiabetic properties, but was too toxic for human use. After hundreds of synthetic compounds were prepared, metformin, a close relative to galegine, was developed and marketed as a useful antidiabetic drug. These examples and many others serve to illustrate the continuing value and importance of plant-derived secondary metabolites as model compounds for modern drug development.(Balandri, Kinghorn and Fransworth, 1993)

1.6 The Depletion of Genetic Resources and the Urgent Need for Conservation Efforts

In spite of impressive recent advances in available extraction technology, separation science (chromatographic techniques), and analytical and spectroscopic instrumentation, we still know surprisingly little about the secondary metabolism of most of the world's higher plant species. This is especially true in the case of tropical rain-forest floras. Although the tropics contain most of the world's plant species, it has been estimated that more than half of these are unknown to science (having never been described) and that most have never been surveyed for chemical constituents.

For example, it has been estimated that nothing is known about the chemistry of the vast majority of the plant species comprising the immense flora. This paucity of knowledge is alarming in view of the current rate of extinction and decimation of tropical floras and ecosystems, especially forests, before their plants have been adequately catalogued and studied.

If the current trends of destruction of tropical forest habitats and general global simplification of the biota continue at their present rates, biochemists, ethnobotanists, molecular biologists, organic chemists, pharmacognosists, pharmacologists, taxonomists, and other scientists interested and involved in medicinal plant research may have only a few decades remaining in which to survey and sample the diverse chemical constituents of a large part of the plant kingdom for potentially useful novel bioactive compounds. Under such circumstances, it is virtually certain that many significant opportunities for successful drug development will be lost. (Balandri, Kinghorn and Fransworth, 1993)

1.7 Prospects for the Future of Plant-Derived Natural Products in Drug Discovery and Development

In spite of numerous past successes in the development of plant-derived drug products, it has been estimated that only 5 to 15% of the ca. 250,000 existing species of higher plants have been systematically surveyed for the presence of biologically active compounds. Moreover, it is often the case that even plants that are considered to have been "investigated" have been screened for only a single type (or, at best, a few types) of biological activity. The best example of an extensive, but narrow, screening program is the National Cancer Institute's search for antitumor agents from higher plants.

Over a 25-year period, an enormous number of plant extracts representing approximately 35,000 plant species were tested solely for cytotoxic and antitumor activity using only a few different bioassays. The hard-won successes of this massive screening effort are now apparent in the successful development of anticancer agents such as taxol and derivatives and camptothecin and analogues. However, during the course of this screening effort, naturally occurring compounds potentially useful as new drugs for other ailments or conditions (e.g., analgesic, antiarthritic, antipsychotic, and psychotropic agents) were overlooked.

Thus, since at least 85% of the world's species of higher plants have not been adequately surveyed for potentially useful biological activity, it appears that the plant kingdom has received relatively little attention as a resource of potentially useful bioactive compounds. Because many plant secondary metabolites are genus- or species-specific, the chances are therefore good to excellent that many other plant constituents with potentially useful biological properties remain undiscovered, uninvestigated, and undeveloped. Furthermore, there is the hope that in the future, the process of plant drug discovery and development by way of mass screening will be greatly facilitated and made more efficient by using new automated multiple biological screening methods which are now becoming available and which require only minimal amounts of test samples for evaluation. (Balandri, Kinghorn and Fransworth, 1993)

1.8 Drug Discovery from Natural Resources: Advantages and Disadvantages

Usage of botanical sources as starting point in the drug development program is associated with few specific advantages:

- Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active com-pounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. At certain time point afterward, one may attempt upon synthesis of active molecule and reduce pressure on the resource. Drug development from *Rauwolfia serpentina*, *Digitalis purpurea*, etc. in the past fall under this category of approach.

- Sometimes, such approaches lead to development of novel molecules derived from the source due to inherent limitations of the original molecule. For instance, podophyllin derived from *Podophyllum hexandrum* was faced with dose-limiting toxicities. Such limitations could be overcome to a great extent by semi-synthesis of etoposide, which continues to be used in cancer therapy today. Similar was the case with camptothecin (originally isolated from *Camptotheca sp*. and subsequently from *Mappia sp*.), which led to development of novel anticancer molecules like topotecan and irinotecan.

- Natural resources as starting point has a bilateral promise of delivering the original isolate as a candidate or a semi-synthetic molecule development to overcome any inherent limitations of original molecule.

On the other hand, drug development from natural resources is also associated with certain disadvantages:

- More often than not, drug discovery and eventual commercialization would pressurize the resource substantially and might lead to undesirable environmental concerns. While synthesis of active molecule could be an option, not every molecule is amenable for complete synthesis. Hence, certain degree of dependence on the lead resource would continue. For instance, anticancer molecules like etoposide, paclitaxel, docetaxel, topotecan, and irinotecan continue to depend upon highly vulnerable plant resources for obtaining the starting material since a complete synthesis is not possible. On the other hand, it is expected that some 25,000 plant species would cease to exist by the end of this century.

- Over a period of time, the intellectual property rights protection related to the natural products is going haywire. By and large, the leads are based upon some linkage to traditional usage. With larger number of countries becoming the parties to the Convention on Biological Diversity (CBD), the process of accessing the basic lead resource, benefit sharing during the commercial phase, etc. became highly complex in many countries. These processes tend to impede the pace of discovery process at various phases irrespective of the concerns leading to such processes. (Katiyar *et al*., 2012)

1.9 *Syzygium samarangense* Plant

Taxonomy(Khandaker, 2015)

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Super division	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Rosidae
Order	: Myrtales
Family	: Myrtaceae
Genus	: Syzygium
Species	:*Syzygium samarangense*

Figure 1.14.*Syzygium samarangense* Plant

1.9.1Local Names

English (Wax-apple, water apple, malay apple, Java apple); Filipino (makopa); Indonesian (jambu klampok); Malay (jambu air mawar); Thai (chomphu-khieo); Vietnamese (roi).(Khandaker, 2015)

1.9.2 Description

The wax apple (*Syzygium samarangense)* is a non-climacteric tropical fruit species. It is also known as wax apple, rose apple or java apple. Its pear-shaped fruits are usually pink, light-red, red, green, sometimes greenish-white, or cream-colored and are generally crisp, with a subtly

sweet taste or aromatic flavor.The species presumably originated in Malaysia and other South-east Asian countries. It is widely cultivated and grown throughout Malaysia and in neighboring countries such as Thailand, Indonesia and Taiwan.

There are three species of Syzygium, namely the water apple *(Syzygium aquem)*, Malay apple (*Syzygium malaccense*) and wax apple (*Syzygium samaragense*) bear edible fruits. Wax apple contains fruit with more round and oblong in shape and less watery compare to the other Syzygium speciesand the fruits are eaten raw with salt or cooked as sauce.

It is a very delicious fruit to eat, sweet soft and watery. Despite its name, a ripe wax apple only resembles an apple on the outside in color. It doesn't taste like an apple, and it has neither the fragrance nor the density of an apple. Its flavor is similar to a snow pear, and the liquid to flesh ratio of the wax apple is comparable to a watermelon. Unlike either apple or watermelon, the wax apple's flesh has a very loose weave. The very middle holds a seed that's situated in a sort of cotton-candy-like mesh. This mesh is edible but flavorless. The color of its juice depends on the cultivar of the fruit; it may be purple to entirely colorless. As well as in its native range, it is also cultivated in tropical regions such as Vietnam, Taiwan, Thailand, and Bangladesh. (Khandaker, 2015; Alternative Medicine, 2016)

1.9.3 Morphological Description and Their Medicinal Uses

Tree

The tree is 16 to 50 ft (5-15 mt) tall, has a short trunk 10 to 12 in (25-30 cm) thick, and open, widespreading crown, and pinkish-gray, flaking bark.

Leaves

The opposite leaves are nearly sessile, elliptic-oblong, rounded or slightly cordate at the base; yellowish to dark bluish-green; 4 to 10 in (10-25 cm) long and 5-12 cm wide; very aromatic when crushed. It is used as astringent, to treat fever and halt diarrhoea. Powdered leaves are used for cracked tongues. Juice of leaves is used in baths and lotion. It is also used in diabetes, cough and headaches.

Flowers

Flowers, borne in drooping panicles of 3 to 30 at the branch tips or in smaller clusters in the axils of fallen leaves, are fragrant, yellowish-white, 3/4 to 1 1/2 in (2-4 cm) broad, 4-petalled, with numerous stamens 3/5 to 1 in (1.5-2.5 cm) long.

Fruites

The waxy fruit, usually light-red, sometimes greenish-white or cream-colored, is pear-shaped, narrow at the base, very broad, flattened, indented and adorned with the 4 fleshy calyx lobes at the apex; 3.4-5 cm long, 4.5-5.4 cm wide. The skin is very thin, the flesh white, spongy, dry to juicy, sub acid and very bland in flavor. There may be 1 or 2 somewhat rounded seeds0.5-0.8 cm wide, or none. It is used in diabetes, stomatitis aphtosa, diuretic, emmenagogue, abortifacient and febrifuge. Decoction of fruits is used in fever.

Seeds

Each fruit contains a single large, subglobose seed or a pair of subglobose to hemispherical seeds 1.6–2 cm (0.6–0.8 in) in diameter, light brown externally, green internally, and somewhat meaty in texture. The fruits of some trees are entirely seedless.

Root-bark and Root

Each fruit contains a single large, subglobose seed or a pair of subglobose to hemispherical seeds 1.6–2 cm (0.6–0.8 in) in diameter, light brown externally, green internally, and somewhat meaty in texture. The fruits of some trees are entirely seedless.

Bark and Stem

Juice of bark is used to treat wounds and the bark is used as astringent in mouthwash preparations for the treatment of thrush. Decoction of stem is used to treat diarrhea.

(Peter *et al*, 2011)

Figure 1.15. *Syzygium samarangense* (wax jambu) fruit and flower

1.9.4 Edible Uses

It is a very delicious fruit to eat, sweet soft and watery. Despite its name, a ripe wax apple only resembles an apple on the outside in color. It doesn't taste like an apple, and it has neither the fragrance nor the density of an apple. Its flavor is similar to a snow pear, and the liquid to flesh ratio of the wax apple is comparable to a watermelon. Unlike either apple or watermelon, the wax apple's flesh has a very loose weave. The very middle holds a seed that's situated in a sort of cotton-candy-like mesh. This mesh is edible but flavorless. The color of its juice depends on the cultivar of the fruit; it may be purple to entirely colorless. As well as in its native range, it is also cultivated in tropical regions such as Vietnam, Taiwan, Thailand, and Bangladesh. (Alternative medicine, 2016)

1.9.5 *Syzygium samarangense* Leaf

The chemical composition of the leaf essential oils of *Syzygium samarangense* at young, mature, and old stages was analyzed through gas chromatography–mass spectrometry. There were 33 compounds identified. The main components of 3 stages of leaf essential oils were α-pinene, γ-terpinene, β-caryophyllene, α-selinene, β-selinene, and selina-6-en-4-ol. The results demonstrated that levels of β-elemene, γ-terpinene, and β-caryophyllene decreased during maturation. However, caryophyllene oxide and p-cymene gradually increased. The mature stage oil exhibited higher level of monoterpene which may be associated with the potent superoxide scavenging effect but exhibited lower level of sesquiterpene compare to other stages of essential

oils. The old stage essential oil exhibited more pleasant fruity aroma which may be related to increases of p-cymene, caryophyllene oxide, and trans-3-caren-2-ol**. (**Lee *et al*., 2016)

Figure 1.16. *Syzygium samarangense leaves*

1.9.5.1 Industrial relevance and Use

The remarkable radicals scavenging effect and aroma exhibited by the leaf oil can be incorporated into the food, drug or cosmetic formulations to reduce the risk of cancer, cardiovascular diseases or skin ageing and to increase flavor of foods or cosmetics. We are currently focus on investigating the application of extracts of *Syzygium samarangense* on skin care and on antimicrobial activity in cosmetic industry.

Essential oil from the leaf of *Syzygium samarangense* can inhibit Escherichia coli growth by reducing its extracellular protease. 5 However, little or no study conducted on the chemical composition of the leaf essential oils of S. samarangense during maturation. Chemical compositions and biological activities at various stages of maturation plant tissues may have a significant difference. Artemisia annua essential oils exhibited high levels of monoterpene at preflowering phase (89.11%). By contrast, high levels of sesquiterpenes were obtained at postflowering phase (58.73%). 6 It has been reported that various flowering stages of Artemisia annua essential oils exhibited distinct chemical compositions and antiacetylcholinesterase activities. (Alternative medicine, 2016)

Chapter Two
Literature Review

Literature Review

2.1. *Syzygium samarangense:*Phytoconstituents

Investigators have found their principal constituent to be tannins in *Syzygium samarangense* plant.

Leaves contain-

- Lupeol (triterpenoid)
- Betulin (triterpenoid)
- Epi-betulinicacid (triterpenoid)
- 2, 4-dihydroxy-6-methoxy-3-methylchalcone
- 2-hydroxy-4, 6-dimethoxy-3-methylchalcone
- 2, 4-dihydroxy-6-methoxy-3, 5-dimethylchalcone
- 2, 4-dihydroxy-6-methoxy-3-methyldihydrochalcone
- 7-hydroxy-5-methoxy-6, 8-dimethylflavanone
- 2-hydroxy-4, 6-dimethoxy-3-methyldihydrochalcone
- 2, 4-dihydroxy-6-methoxy-3, 5-dimethyldihydrochalcone
- Sitosterol
- Alpha-carotene and Beta-carotene
- Leaf oil is largely composed of monoterpenes (30% sesquiterpenes, 9 % caryophyllene).

Aerial parts contain-

- Ursolic acid
- Jacoumaric acid and Arjunolic acid
- Mearnsitrin,
- 2 - C-Methyl-5-*O*-Galloylmyricetin-3-*O*-α-l-Rhamnopyranoside
- Desmethoxymatteucinol,
- 4, 6 Dihydroxy-2-Methoxy-3
- 5-Dimethylchalcone

- Methyl 3-*epi*-betulinate
- Oleanolic acid
- Desmethoxymatteucinol
- 5-*O*-Methyl-4-desmethoxymatteucinol
- Oleanic acid
- Quercetin glycosides are also present in this plant which includes Reynoutrin, Hyperin, Myricitrin, Quercitrin, Quercetin, Guaijaverin. It also contain Flavanone - (*S*)-pinocembrin, and phenolic acids (Gallic acid and Ellagic acid). (Lee *et al*., 2016)

2.2.*Syzygium samarangense*: Pharmacological Studies

- **Antiinflammatory Activity**

Flavan-3-ols isolated from some medicinal plants inhibiting COX-1 and COX-2 catalyzed prostaglandin biosynthesis. *S samarangense* was one of four plants tested that were traditionally used for inflammatory conditions.

- **Antioxidant Activity**

Study of 58 underutilized Malaysian fruits of 32 different species, showed fruits from some genera, including Syzygium, had higher antioxidant capacity compared to other genera.

A methanolic extract of fresh leaves exhibited high antioxidant activity with DPPH and hydroxyl radical scavenging assays. A strong correlation was noted with phenolic and flavonoid contents. Study of both extracts of ripe and unripe rose apple fruits showed high antioxidant activity and free radical scavenging. However, the unripe rose apple showed greater activity.

- **Leaf oil analysis**

Study of hydrodistilled essential oil from the fresh leaves of *Syzygium samarangense* grown in Nigeria showed the oil to be largely composed of monoterpenes (61.1%) characterized mainly by a-pinene, b-pinene, p-cymene and a-terpineol. The sesquiterpenes constituted 30.8% of the oil with b-caryophyllene as the major component.

- **Essential oils and ichthyotoxicity**

Preliminary ichthyotoxic test on all parts of *Syzygium samarangense* showed the leaves fraction to be most ichthyotoxic against tilapia fish (*Tilapia oreochromis*). Study isolated three compounds – ursolic acid, B-sitosterol, and sitos-4-en-3-one. None of the compounds gave any significant ichthyotoxicity.

- **Aldose Reductase Inhibition or Cataract Prevention**

Cataractogenesis is a common complication in diabetes, and aldose reductase in a lens enzyme involved in its development. In a study, *Syzygium samarangense*was one of the best four plant extract inhibitors with a preventive effect on cataract formation.

- **Hypoglycemic and Antidiabetic activity**

Study of *S. samarangense*aqueous and alcoholic bark extracts in STZ-induced diabetic rats showed reduction of blood sugar and improvement in hyperlipidemia and liver glycogen depletion. The alcohol extract was more effective than the aqueous extract and equivalent to that of glibenclamide.

- **Hypoglycemic and Hypolipidemic activity**

Study evaluated the effect of aqueous and alcoholic extracts of *S. samarangense*on serum glucose, lipid profile and liver glycogen in normal and hyperglycemic rats. Results showed reduction of FBS and significant reversal of diabetes induced hyperlipidemia and liver glycogen depletion.

- **Anticancer Effect**

Study evaluated the antiproliferative effects of three Syzygium fruits, viz., water apple, milk apple, and malay apple against two types of cancer-origin cells MCF-7 (hormone dependent breast cancer cell line) and MDA-MB-231 (nonhormone-dependent breast cancer cell line). The extracts of water apple and malay apple displayed antiproliferatiion effects on MCF-7 cell lines in 72 hours. The methanol extract of S. malaccense showed a more significant effect.Study

showed malay apple extract showed anti-proliferation effects on MCF-7 cell lines in 72 hours. A methanolic extract showed 79% cell viability of MCF7.

- **Subacute Toxicity Testing or Hematological and Liver Tissue Effects**

Study evaluated the subacute effects of extract of *S. samarangense*in albino rats in doses of 50, 100, 250, 500 mg/kbw for 28 days. Results showed a tendency to affect the hematopoietic elements and alter the structural integrity of the liver tissue if ingested at higher doses.

- **Calcium Antagonist or Anti-Diarrheal Activity**

Study evaluated the use of the plant in hypermotility states of the gut. A hexane extract was found to dose-dependently relax the spontaneously contracting isolated rabbit jejunum. Four flavonoids isolated from the hexane extract showed dose dependent spasmolytic activity with SS2 showed the most potency. These compounds exhibiting spasmolytic and calcium antagonist activity may be responsible for the medicinal use of the plant in diarrhea. (Stuart Jr., 2015)

2.3. Study on "Physiological and biochemical properties of three cultivars of wax apple (*Syzygium samarangense*) fruits."

A study under field condition was carried out to evaluate physiological and biochemical properties of three cultivars of *Syzygium samarangense* namely 'Jambu Madu Red', 'Masam Manis Pink' and 'Giant Green'. Physicochemical parameters, such as stomatal conductance, fruit development, pigmentation, fruit shape, yield, total soluble solids, titratable acidity, sugar acid ratio, vitamin-C, chlorophyll, carotene and anthocyanin content, in three cultivars of wax apple were investigated. The highest stomatal conductance, color development and yield were recorded in 'Jambu Madu Red' cultivar. Lowest amount of titratable acidity, highest total soluble solids, sugar acid ratio and carotene content were also observed in this cultivar.

Earlier color development, fruit maturity, good shape, highest vitamin-C and anthocyanin content were found in 'Masam Manis Pink'. Meanwhile, the highest chlorophyll *a*, *b* and total chlorophyll content and late maturation fruit were recorded in 'Giant Green' Cultivar. Stomatal

conductance showed positive correlation with yield and fruit biomass. It is concluded that 'Jambu Madu Red' and 'Masam Manis Pink' cultivars are comparatively better yield than those of 'Giant Green' cultivar grown under tropical field conditions. (Hossain *et al*., 2015)

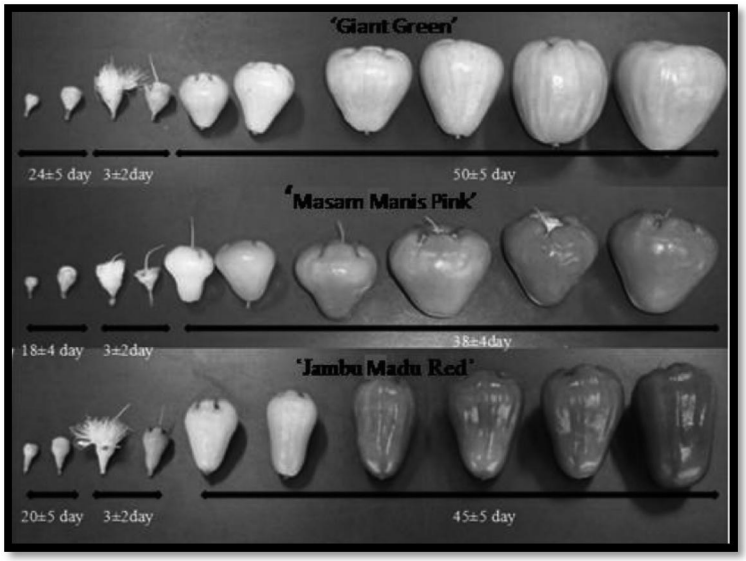


Figure 2.1. Fruit development and ripening of different cultivars of *Syzygium samarangense.* (Hossain *et al*., 2015)

2.4. Study on "Evaluation of analgesic, anti-inflammatory and CNS activities of the methanolic extract of *Syzygium samarangense* leaves"

Syzygium Samarangense is a potential medicinal drug. This study aimed to evaluate the analgesic, anti-inflammatory and CNS activities of the methanolic extract of *Syzygium Samarangense* leave in mice. The analgesic activity was examined by acetic acid induced writhing and formalin tests. The anti-inflammatory activity was studied using carrageenan induced hind paw edema model. The analgesic activity of the methanolic extract of *Syzygium Samarangense* leaves was evaluated by acetic acid induced writhing and formalin tests at the dose of 100 mg/kg and 200 mg/kg, significantly (p<0.05) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner

The extract of *SyzygiumSamarangense* leaves caused significant (p <0.05) inhibition of carrageenan induced paw edema after 4 hrs in a dose dependent manner. The CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the open field and hole cross tests at a dose of 100 mg/kg and 200 mg/kg body weight. The findings of the study suggested that the methanolic extract of *Syzygium Samarangense* leave has remarkable analgesic, moderate effect against inflammation and significant CNS effects, conforming the traditional use of this plant for inflammatory pain alleviation. (Mollika *et al*., 2014)

2.5. Research on "Chemical composition of leaf essential oils of *syzygium samarangense* at three maturity stages"

The chemical composition of the leaf essential oils of *Syzygium samarangense*. Pink at young, mature, and old stages was analyzed through gas chromatography–mass spectrometry (GC-MS). There were 33 compounds identified. The main components of 3 stages of leaf essential oils were α-pinene, γ-terpinene, β-caryophyllene, α-selinene, β-selinene, and selina-6-en-4-ol. The results demonstrated that levels of β-elemene, γ-terpinene, and β-caryophyllene decreased during maturation. However, caryophyllene oxide and p-cymene gradually increased. The mature stage oil exhibited higher level of monoterpene which may be associated with the potent superoxide scavenging effect but exhibited lower level of sesquiterpene compare to other stages of essential oils. The old stage essential oil exhibited more pleasant fruity aroma which may be related to increases of p-cymene, caryophyllene oxide, and trans-3-caren-2-ol**.**

2.5.1. Extraction yields

The essential oils obtained from young, mature, and old leaves of *S. samarangense* produced 0.02%, 0.03%, and 0.02% yield (v/w), respectively.

2.5.1.1. Chemical composition of essential oils

Thirty-three components have been identified at each development stage of the leaf essential oils.

The main components in the three leaf essential oils were α-pinene (21.85%–26.40%), γ-terpinene (9.51%–11.17%), β- caryophyllene (4.67%–7.72%), α-selinene (4.67%–5.60%), β-selinene (4.94%–6.00%), and selina-6-en-4-ol (5.07%–5.21%). Trans-3-caren-2-ol was only found in mature and old leaf essential oils. However, germacrene D was only found in young leaf essential oil. Germacrene D is considered to be a precursor to many sesquiterpene hydrocarbons and that may be associated with lower levels of sesquiteterpene in mature and old leaves.

Various changes to the chemical compositions of leaf essential oils during maturation were observed in the studies. Monoterpene increase in young leaves from 42.85% to 50.34% in mature leaves, and may be related to the increase of α-pinene from 21.85% to 26.40%. Sesquiterpene decrease from 32.06% to 22.06%, which may be related to reduction of β-elemene and β-caryophyllene.

In this study, the β-caryophyllene content in young leaf essential oil was higher than in mature and old leaf essential oils. β-caryophyllene was oxidize into caryophyllene oxide over time. Furthermore, β- caryophyllene induced chlorosis in leaves. Therefore, the amount of normal growth β-caryophyllene in leaves decrease and transforme into caryophyllene oxide over time. Caryophyllene oxide is frequently used in cosmetics and as a flavoring agent in foods. Therefore, increasing caryophyllene oxide in old leaf essential oil may contribute to its more mild and pleasant odor than in young leaf essential oil.

The other monoterpene γ-terpinene can aromatize into p-cymene, which is widely used as an intermediate in the synthesis of fine chemicals for flavorings, fragrances, perfumes, fungicides, and pesticides. γ-terpinene decrease during maturation and the amount of p-cymene gradually increase, which indicate that γ-terpinene transforme to p-cymene at the mature and old leaf stages.

Trans-3-caren-2-ol is only found in mature and old leaf essential oils. Car-3-ene has been reported to contribute to the characteristic mango odor of the rhizome of Curcuma amada Roxb and the Venezuelan mango. Trans- 3-caren-2-ol also cause the fruity aroma of mature and old leaves. Therefore, the pleasant fruity aroma of old leaf essential oil may be related to p-cymene, caryophyllene oxide, and trans-3-caren-2-ol.(Lee *et al*. 2016)

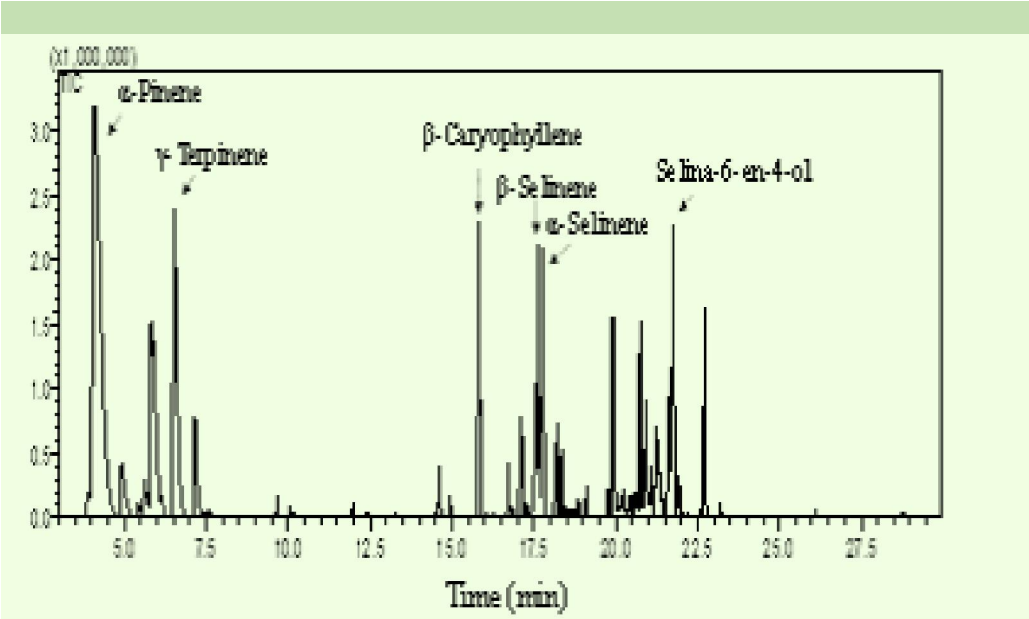
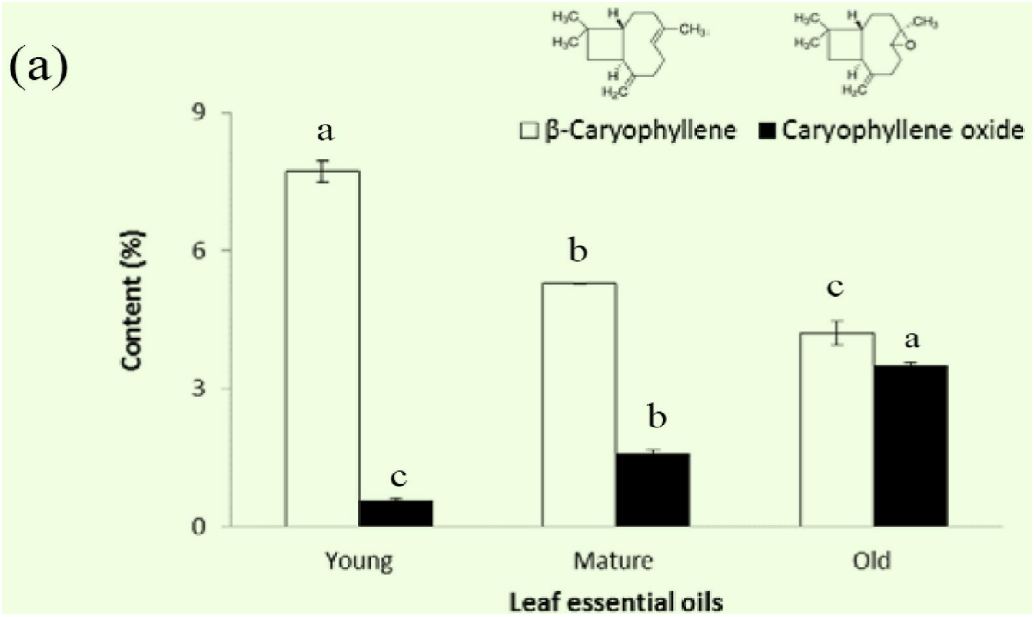


Figure 2.2. The main components of old *Syzygium samarangense* leaf essential oils by GC-MS analysis.



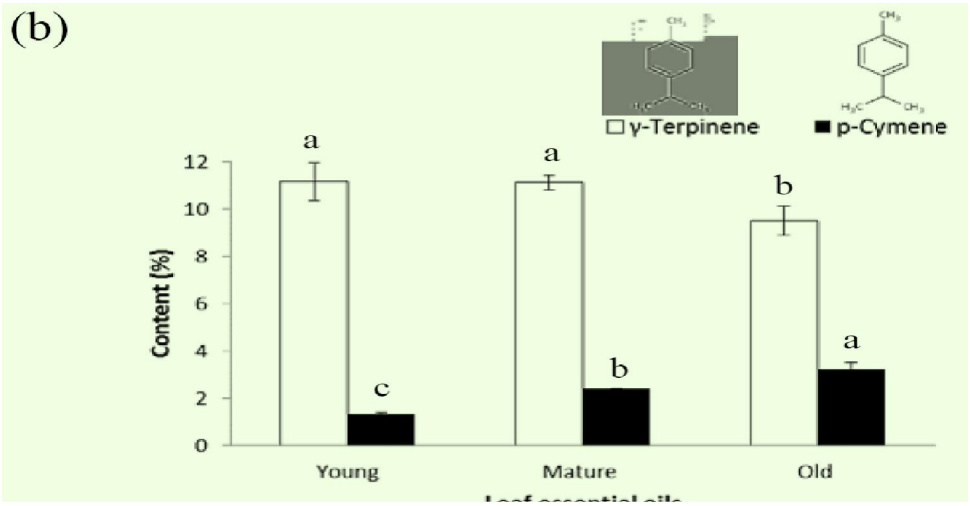


Figure 2.3. Content of the different components of *Syzygium samarangense* leaf essential oils at 3 different maturity stages.

(a) Content of the β-caryophyllene and caryophyllene oxide of young, mature and old oils.

(b) Content of $_{\gamma}$-terpinene and *p*-cymene of young, mature and old oils. The same component of three different maturity oils with different lower case letters indicates a significant difference.

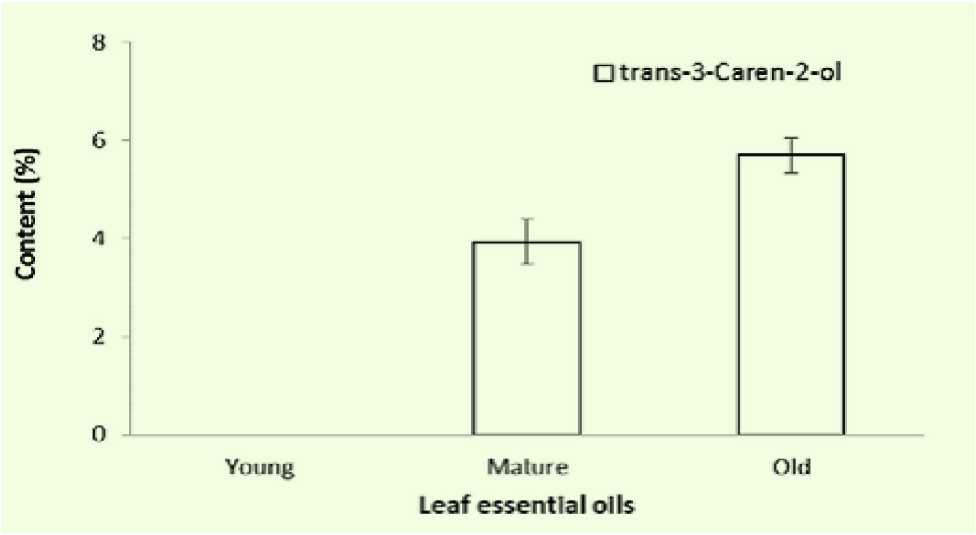


Figure 2.4. The content of trans-3-caren-2-ol of *Syzygium samarangense* leaf essential oils at 3 different maturity stages. The component of three different maturity oils with different lower case letters indicates a significant difference.(Lee *et al*. 2016)

2.6. Study on "Anti-proliferative and apoptotic activities of *syzygium samarangense* fruits extract against human A549 lung cancer cell lines."

Medicinal plants have been in use from time immemorial and their utility has been expanding step by step in the present world. The intention of the study was to check the anti-proliferative and apoptotic activity of S*yzygium samarangense* fruits methanolic extract against of A549 cell lines.

The methanol extract at different concentrations were tested against A549 human lung cancer cell lines for cell viability or cytotoxicity by MTT assay and the hallmark of the apoptosis was analyzed by DNA fragmentation method. The morphological changes resulted due to apoptosis were investigated by Propidium Iodide (PI) staining technique.

The results showed that the tested extracts showed strong and decreased cell viability in a concentration-dependent manner. IC_{50} value represented that the anti-proliferative activity was found with a minimum concentration of 21.86µg/ml. The presence of ladders of DNA fragments in the DNA fragmentation assay indicates a biochemical hallmark of intrinsic apoptotic cell death. Altered cell morphology after treatment with the extract demonstrated that cells experienced apoptosis.

The present findings encourage further for the isolation and identifying, of active components present in *S. samarangense* fruit pulps to understand the mechanism of action *in vivo* that can make an allowance for developing chemotherapeutic agent against cancer.(Thampi and Shalini, 2015)

2.7. Investigation on "Chemical constituents of *syzygium samarangense.*"

The dichloromethane extract of the leaves of *Syzygium samarangense* afforded 2,4- dihydroxy-6-methoxy-3-methylchalcone (1), 2,4-dihydroxy-6-methoxy-3,5-dimethylchalcone (2), 2-hydroxy-4,6-dimethoxy-3-methyl chalcone (3), squalene (4), betulin (5), lupeol (6), sitosterol (7), and a

mixture of cycloartenyl stearate (8a), lupenyl stearate (8b), β-sitosteryl stearate (8c), and 24-methylenecycloartenyl stearate (8d). The structures of 1-3, 5, 7, and 8a-8d were elucidated by 1D and 2D NMR spectroscopy. Sample 8 was tested for hypoglycemic and antimicrobial potentials. It showed negative hypoglycemic potential and exhibited moderate antifungal activity against *C. albicans,* low activity against *T. mentagrophytes* and low antibacterial activity against E. coli, S. aureus and P. aeruginosa. It was inactive against *B. subtilis* and *A. niger*.(Ragasa *et al*., 2014)

2.8. Study on "In vivo evaluation of the pharmacological activities of *Syzygium samarangense*"

Syzygium samarangense is very popular and usually used in the traditional system of medicine. These studies were carried out to investigate the analgesic, anti-inflammatory, CNS depression as well as the anti-diarrheal activity of the methanolic extract of *Syzygium samarangense* leaves (MSSL) and barks (MSSB). MSSL and MSSB were used to investigate the analgesic effect by acetic acid induced writhing and formalin induced licking method whereas carrageenan induced inflammation was used for anti-inflammatory activity.

CNS depression activities were evaluated in hole-cross and open field test methods. Furthermore castor oil-induced diarrhea and charcoal-induced gastrointestinal motility were used to investigate the anti-diarrheal activity of MSSL and MSSB. MSSB at doses of 100, 200 and 300 mg/kg body weight, significantly reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. At 400 mg/kg doses of MSSB showed highest anti-inflammatory activity (% inhibition 72.82%) after 4 hrs. A statically significant CNS depression activity was also observed in both hole cross and open field tests in a dose dependent manner. MSSL and MSSB significantly reduced the frequency and severity of diarrhea in test animals throughout the study period in a dose dependent manner and also showed a significant reduction in the gastrointestinal motility in charcoal meal test.

Altogether, these results suggest that the *Syzygium samarangense* have good pharmacological effects and provide as a part of scientific support for the use of this species in traditional medicine.(Majumder and Alam, 2014)

2.9. Research on "*Syzygium samarangense*: a review on morphology, phytochemistry & pharmacological aspects."

This review gives an account of the current knowledge on Morphology, Phytochemistry and Pharmacological aspects of *Syzygium samarangense*, also known as Java apple, Wax apple is a tropical tree growing to 16 to 50 ft. Wide variety of phytoconstituents such as tannins, glycosides, flavonoids and terpenoids were isolated from the aerial parts of the tree.

Leaves and barks of *Syzygium samarangenese* are used for various ailments like cough, cold and amenorrhoea. Fruits are used as stomatitis aphtosa, diuretic, emmenagogue, abortifacient and febrifuge. The phytochemicals in the Java apple tree show some antibiotic action against Staphylococcus aureus, Candida albicans and Mycobacter smegmatis. The plant has been reported to have antibacterial, antidiabetic, antidiarrhoeal and immunostimulant activities. Hence the present article includes detailed exploration of Morphology, Phytochemistry and Pharmacological aspects of *Syzygium samarangense* in an attempt to provide direction for further research. . (Peter *et al*., 2011)

2.9.1. Pharmacological Activities

WHO recognized several Syzygium species were reported to possess antibacterial, antifungal and anti-inflammatory activities. The flavonoids, isolated from *S. samarangense*, were reported to possess antihyperglycemic activity, spasmolytic and immunomodulatory activities.

- **Antidiarrheal Activity**

The hexane extract of *S. samarangense* was found to dose dependently (10-3000 micro g/mL) relax spontaneously contracting isolated rabbit jejunum. When tested for a possible calcium

channel blocking (CCB) activity, the extract (10-1000 micro g/mL) relaxed the high K+-induced contractions and also decreased the Ca^{++} dose-response curves in a dose dependent manner (30-100 micro g/mL), confirming the CCB activity.

The flavonoids isolated from the hexane extract were tested for a possible spasmolytic activity. All flavonoids, showed dose dependent (10-1000 micro g/mL) spasmolytic activity. These indicate that the presence of compounds with spasmolytic and calcium antagonist activity may be responsible for the medicinal use of the plant in diarrhea.

- **Anticholinesterase Activity**

The actual inhibitory assay involves the addition of 30 µL of test sample solution and 30 µL of enzyme stock solution to 2.81 µL of phosphate buffer-1. The mixture was incubated for 5 – 10 min at 25°C. A 100 µL of Ellman's reagentstock solution and 30 µL of substrate stock solution were then added and absorbance at 412 nm was recorded. The control used was physostigmine. The percent inhibition was calculated. When tested against butyrylcholinesterase, it exhibited 68.0% inhibitory activity at 0.20 mM concentration and its IC50 was determined to be 127 µM. The IC50 of physostigmine, the positive control, was 0.041 µM and 0.857 µM against acetylcholinesterase and butyrylcholinesterase respectively.

- **Immunopharmacological Activity**

The flavonoids isolated from *S. samarangense* were evaluated for immune-pharmacological activity. Human peripheral blood mononuclear cells were used as target cells, and cell proliferation was determined by 3H-thymidine uptake. The flavonoids are-

  - (-)-Strobopinin
  - Myricetin 3-O-(2"-O-galloyl)-alpha-rhamnopyranoside
  - (-)- Epigallocatechin 3- O-gallate and
  - Myricetin 3-O-alpha-rhamnopyranoside

These compounds showed inhibitory potency on PBMC proliferation activated by phytohemagglutinin (PHA). The IC50 values of these compounds on activated PBMC proliferation were 36.3, 11.9, 28.9, and 75.6 microM, respectively. The inhibitory mechanisms

may involve the blocking of interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) production, since compounds 1, 2, 3 and 4 reduced IL-2 and IFN gamma production in PBMC in a dose-dependent manner.

- **Cytotoxic Activity**

The flavonoids 2'-hydroxy-4',6'-dimethoxy-3'-methylchalcone, 2',4'- dihydroxy-6'-methoxy-3',5'-dimethylchalcone, 2',4'-dihydroxy-6'-methoxy-3'-methylchalcone, 2',4'-dihydroxy-6'-methoxy-3'-methyldihydrochalcone and 2',4'-dihydroxy-6'-methoxy-3',5'- dimethyl dihydrochalcone, isolated from *Syzygium samarangense*, were subjected to cytotoxicity testing using the dimethyl thiazol diphenyl tetrazolium (MTT) assay. The cell lines used were the Chinese hamster ovarian and the human mammary adenocarcinoma.

Among the test compounds, 2',4'- dihydroxy-6'-methoxy-3',5'-dimethylchalcone exhibited significant differential cytotoxicity against the MCF-7 (Michigan Cancer Foundation-7, a breast cancer cell)cell line with an IC50 of 0.0015 ± 0.0001 nM. It was also cytotoxic against the SKBR-3 (human mammary adenocarcinoma cell line) cell line with an IC50 of 0.0128 ± 0.0006 nM. Doxorubicin, the positive control, had an IC50 of 2.60 ± 0.28 × 10-4 nM against the MCF-7 cell line and an IC50 of 2.76 ± 0.52 × 10-5 nM against the SKBR-3 cell line.

When tested in a mechanism-based yeast bioassay for detecting DNA-damaging agents using genetically engineered *Saccharomyces cerevisiae* RS322Y (RAD52) mutant strain and (LF15/11) (RAD+) wild type strain, 2',4'- dihydroxy-6'-methoxy-3',5'-dimethylchalcone showed significant selective cytotoxicity against the RAD52 yeast mutant strain. It had an IC12 of 0.1482 nM, as compared with the positive control, streptonigrin, which had an IC12 of 0.0134 nM. Hence, it is a cytotoxic natural product with potential anticancer application.

- **Antihyperglycaemic activity**

2', 4'-Dihydroxy-3', 5'-dimethyl-6'-methoxychalcone, its isomeric flavanone 5-O-methyl-4'-desmethoxymatteucinol and 2'4'-dihydroxy-6'-methoxy-3'- methylchalcone were isolated from the leaves of *S. samarangense* using a bioassay-directed scheme. In an oral glucose tolerance test, at a dosage of 1.0 mg/20 g mouse, 2', 4'-Dihydroxy-3', 5'-dimethyl-6'-methoxychalcone and

5-O-methyl-4'-desmethoxymatteucinol significantly lowered the blood glucose levels (BGLs) in glucose-hyperglycaemic mice when administered 15 min after a glucose load. When co-administered with glucose, only 2', 4'-Dihydroxy-3', 5'-dimethyl-6'-methoxychalcone showed a significant lowering of BGLs 45 minutes after its oral administration. When administered 15 minutes before glucose, none of the flavonoids showed a positive effect. Only 2', 4'-Dihydroxy-3', 5'-dimethyl-6'-methoxychalcone decreased significantly, at alpha = 0.05, the BGLs of alloxan-diabetic mice at t = 90-150 min.

- **Antibactirial activity**

The methanolic and the petroleum ether extracts of *S. samarangense* exhibited significant antimicrobial activity on certain pathogens. The minimum inhibition and minimum bacterial or fungal concentrations were determined by micro dilution method using 96-well microtitre plate method. As the disc dosage level increases the inhibitory effect is also increased. The extracts were proved as strong inhibitors against Gram negative bacteria than Gram positive bacteria.

- **Analgesic and Anti-Inflammatory activity**

Cycloartenyl stearate, lupenyl stearate, sitosteryl stearate, and 24-methylene cycloartanyl stearate from the air-dried leaves of *Syzygium samarangense* exhibited potent analgesic and anti-inflammatory activities at effective doses of 6.25 mg/kg body weight and 12.5 mg/kg body weight, respectively.

It also exhibited negligible toxicity on zebrafish embryonic tissues. There were incidences of mortality upon direct exposure of 24-methylene cycloartanyl stearate to dechorionated embryos, but higher mortality and aberration were observed during intact chorion treatment.

- **Antioxidant activities**

Antioxidant activity of *S. samarangense* were investigated in fruits. For this, at first matured fruits of them were sliced into small pieces and dried in the sun and finally crushed in a grinder to make powder. Ethanolic extracts of fruit powder were prepared using 99.99% ethanol. The antioxidative activities of the extracts were determined according to their abilities of scavenging

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. It was demonstrated that the ethanolic extracts of *S. samarangense* showed antioxidant activity. The IC_{50} of the ethanolic extract *S. samarangense* was 200 micro g/mL. This indicates the fruit is beneficial to human health.(Peter *et al*., 2011)

2.10. Investigation on "Presence of calcium antagonist activity explains the use of *Syzygium samarangense* in diarrhea"

This study was undertaken to rationalize the use of this plant in hypermotility states of the gut. The hexane extract of *S. samarangense*was found to dose-dependently (10–3000 µg/mL) relax the spontaneously contracting isolated rabbit jejunum. When tested for a possible calcium channel blocking (CCB) activity, the extract (10–1000 µg/mL) relaxed the high K^+-induced contractions and also decreased the Ca^{++} dose-response curves in a dose-dependent manner (30–100 µg/mL), confirming the CCB activity.

Four flavonoids isolated from the hexane extract were tested for a possible spasmolytic activity. All flavonoids, identified as 2′-hydroxy-4′, 6′-dimethoxy-3′-methylchalcone, 2′, 4′-dihydroxy-6′-methoxy-3′, 5′-dimethylchalcone, 2′, 4′-dihydroxy-6′-methoxy-3′-methylchalcone and 7-hydroxy-5-methoxy-6, 8-dimethylflavanone, showed dose-dependent (10–1000 µg/mL) spasmolytic activity with 2′, 4′-dihydroxy-6′-methoxy-3′, 5′-dimethylchalcone being the most potent. These results indicate that the presence of compounds with spasmolytic and calcium antagonist activity may be responsible for the medicinal use of the plant in diarrhea.(Ghayur *et al.*, 2006)

2.11. Research on "Hypotriglyceridemic and hypoglycemic effects of vescalagin from pink wax apple (*syzygium samarangense*) in high-fructose diet-induced diabetic rats."

Vescalagin, an active component from Pink wax apple (*Syzygium samarangense*) fruit, with glucose uptake enhancing ability in insulin-resistant FL83B mouse hepatocytes, also has

hypotriglyceridemic and hypoglycemic effects in high-fructose diet (HFD)-induced diabetic rats. Wistar rats were fed HFD for 16 weeks and orally administered with vescalagin from Pink wax apple daily during the last 4 weeks. The results of biochemical parameters showed that fasting blood glucose, C-peptide, fructosamine, triglyceride and free fatty acid contents decreased by 44.7%, 46.2%, 4.0%, 42.5%, and 10.8%, respectively, in the HFD-induced diabetic rats administered with vescalagin at 30 mg/kg body weight in comparison with those of control HFD-induced diabetic rats.

However, high-density-lipoprotein-cholesterol content increased by 14.4% in the HFD rats treated with vescalagin. The study reveals that vescalagin could have therapeutic value against diabetic progression via its anti-hypertriglyceridemic and anti-hyperglycemic effects. (Shen and Chang, 2013)

Chapter Three
Methods and Materials

Methods and Materials

3.1 Collection and preparation of plant material

Plant sample of *Syzygium samarangense*was collected from Narayangonj in March, 2016. Then proper identification of plant sample was done by an expert taxonomist. The plant was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried plant was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Extraction of the plant material

About 650 gm of the powdered material was taken in separate clean, round bottomed flask (5liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filterpaper and the filtrate thus obtained was concentrated at 390°C with a Heidolph rotary evaporation.

Figure 3.1: Drying of extract using rotary evaporator

The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 25.18 gm respectively.

3.3. Preparation of Mother Solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This is the mother solution.

3.4. Partition of Mother Solution

The mother solution was then partitioned off successively by four solvents of different polarity.

3.4.1. Partition with Pet-ether

The mother solution was taken in a separating funnel. 100 ml of the Pet-ether was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml X 3). The Pet-ether fraction was then air dried for solid residue.

3.4.2 Partition with Dichloromethane

To the mother solution left after partitioning with Pet-ether, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with Dichloromethane (DCM). The process was repeated thrice (100 ml X 3). The DCM fraction wasthen air dried for solid residue.

3.4.3 Partition with Ethyl acetate

To the mother solution that left after washing with Pet-ether, and Dichloromethane, 16 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with ethyl acetate. The process was repeated thrice (100 ml X 3). The ethyl acetate fraction was then air dried for solid residue.

3.4.4 Partition with Aqueous Fraction

After partitioning the mother solution with Pet-ether, Dichloromethane and Ethyl acetate, 20 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with aqueous fraction. The process was repeated thrice (100 ml X 3). The aqueous fraction was then air dried for solid residue.

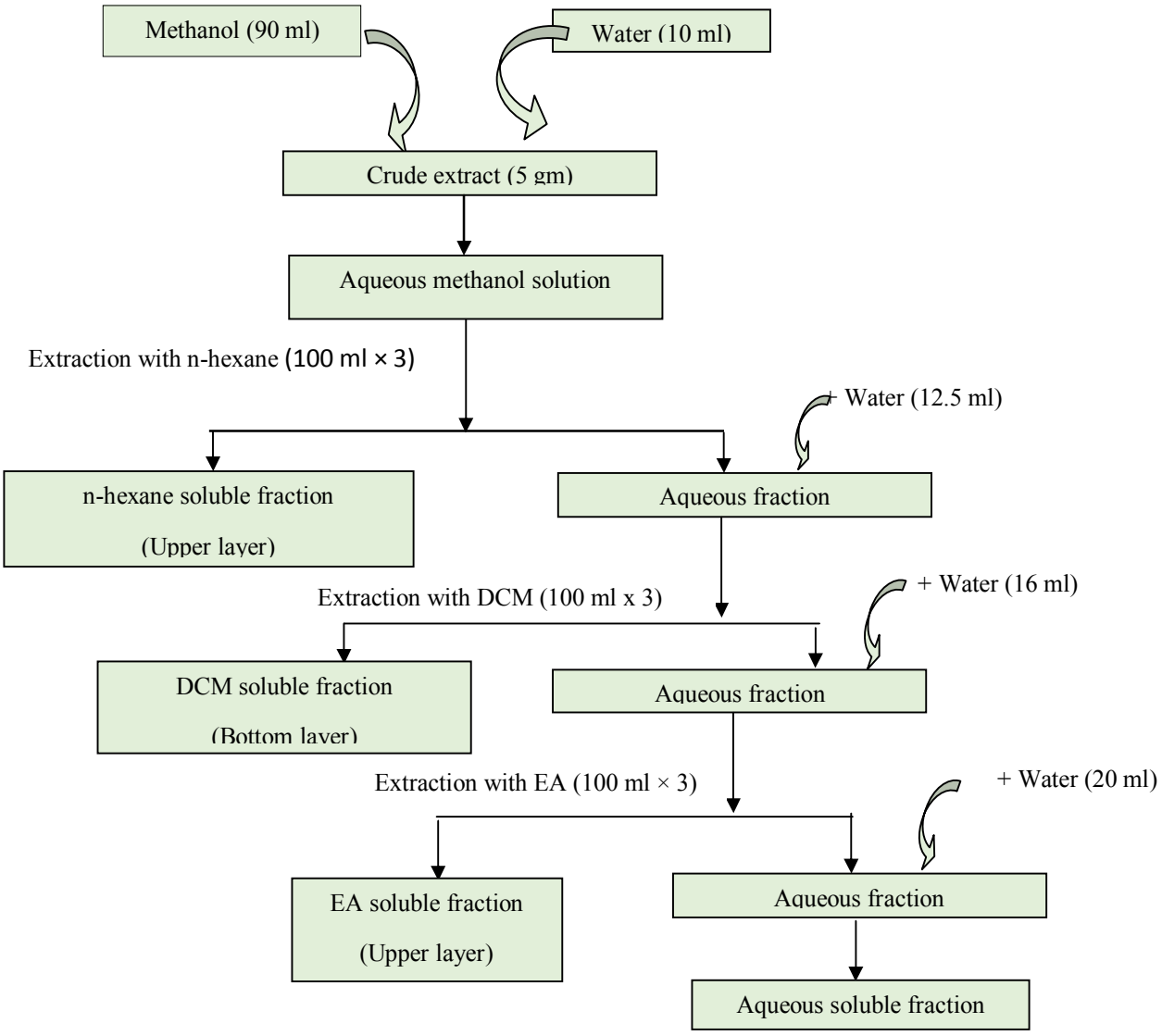


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Sygyzium samarangense.*

3.4.5 Collection of DCM Fraction

After partitioning the mother solution with the four different solvents the DCM fraction of them were collected and air dried. This DCMfraction was further investigated for different pharmacological properties such as Antioxidant and Cytotoxic (Beckett AH and Stenlake JB, 1986).

3.5 Brine Shrimp Lethality Bioassay

3.5.1 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (*in-vivo*) lethality, a simple zoological organism, (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested or their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia* is the only genus in the family Artemiidae. (Olowa *et al.*, 2013).

3.5.2 Apparatus & Reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

Artemia salina leach (brine shrimp eggs)	Pipettes & Micropipette
Sea salt (NaCl)	Glass vials

Small tank with perforated dividing dam to hatch the shrimp	Magnifying glass
Lamp to attract shrimps	Test samples

3.5.3 Procedure

3.5.3.1 Preparation of Sea Water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 38 gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000 ml by distilled water in a 1000 ml beaker for *Artemia salina* hatching. 1-2 drops of 1 N NaOH or 1 N HCl solution was added with a dropper for obtaining the pH 8.4 as sea water.

3.5.3.2 Hatching of Brine Shrimp

A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Then a dry preserved egg of *Artemia salina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemiasalina* were hatched at room temperature (25-30ºC) for 18-24 hours. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5 ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay (Niazi J. *etal*., 2009).

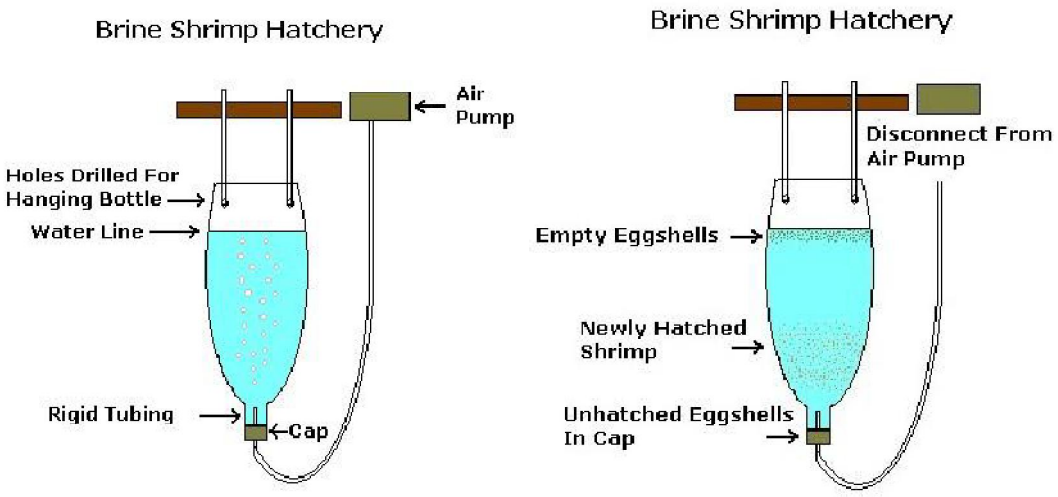


Figure 3.3: Brine shrimp Hatchery.

3.5.3.3 Preparation of Test Solutions

Clean test tubes were taken. These test tubes were used for ten different concentrations (one test tube for each concentration) of test samples and ten test tubes were taken for standard drug tamoxifen for ten concentrations of it and another one test tube for control test.

3.5.3.4 Preparation of the Test Samples of Experimental Plant

All the test samples of 4 mg were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions. Then 100 µl of solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100 µl DMSO was added to vial. Thus the concentrations of the obtained solution in each test tube were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml for 10 dilutions.

3.5.3.5 Preparation of the Positive Control Group

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 2000 µg/ml. From that stock solution serial dilutions are made using DMSO to get 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml. Then ten living brine shrimp nauplii in 5 ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.5.3.6 Preparation of the Negative Control Group

100 µl of DMSO was added to the pre-marked test tube containing 5 ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds (Goldstein *et al.*, 1974).

3.5.3.7 Counting of Nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration (Sleet RB and Brendel K, 1983).

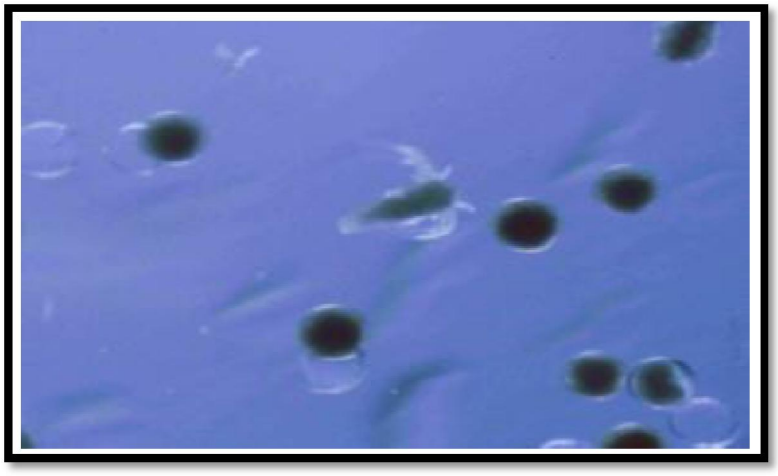


Figure 3.4: Counting of nauplii

3.6 Antioxidant Activity

3.6.1 Total Phenolic Content

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the antioxidative status occurrences of human diseases. In addition, antioxidant compounds which are responsible for such antioxidant activity could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidant effects have rarely been carried out. The purpose of this study was to evaluate extractives of*S.samarangense* new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity.

3.6.1.1 Principle

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. In the alkaline condition phenols ionize completely.

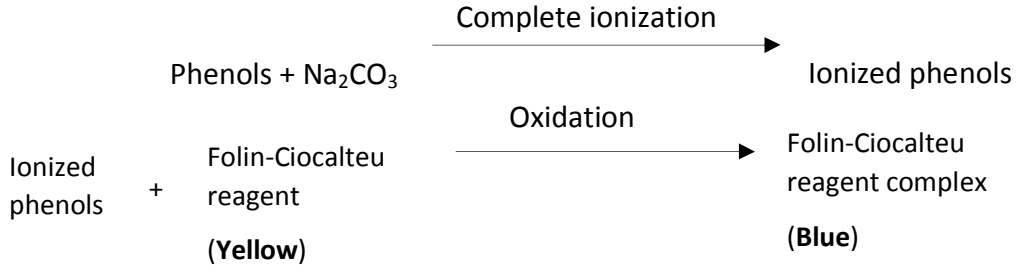
Table 3.2: Composition of 100 mg Folin-Ciocalteu Reagent

Composition of 100 mg Folin-Ciocalteu Reagent	
Water	57.5 ml
Sodium Tungstate Dihydrate	10.0 mg

Hydrochloric Acid (25%)	10.0 mg
Phosphoric Acid 85% solution in water	5.0 mg
Molybdic Acid Sodium Dihydrate	2.5 mg
Lithium Sulfate	15 mg

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain hetero-polyphosphotunstates - molybdates. Sequences of reversible oneor two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.

The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton et al., 1999).



3.6.1.2 Apparatus & Reagents

Table 3.3: Apparatus and reagents used for total phenolic content

Folin-Ciocalteu reagent (10 fold diluted)	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Na_2CO_3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200 µl)

Distilled water	Cuvette

3.6.1.3 Procedure

Standard curve preparation

Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120 µg/ml to 80 µg/ml. 5 ml of FCR (diluted 10 times with water) and 4 ml of Na_2CO_3 (7.5% w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

Sample preparation

2 mg of the *S.samarangense*DCM fraction was taken and dissolved in 1 ml methanol to get a sample concentration of 2 mg/ml.

Determination of total phenol content

- 1.0 ml plant extract of different concentrations (120 µg/ml, 110 µg/ml, 100 µg/ml, 90 µg/ml and 80 µg/ml) was taken in test tubes.
- 5 ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
- 4 ml of Sodium carbonate solution was added into the test tube.
- The test tubes containing the samples were incubated for 1 hour at the room temperature to complete the reaction.
- Absorbance of solution was measured at 765 nm using a spectrophotometer against blank.

- A typical blank solution containing methanol was taken.

3.6.2 Total Flavonoid Content

3.6.2.1 Principle

Aluminium chloride ($AlCl_3$) colorimetric method is incorporated to determine the total flavonoid contents of the crude plant extract. The basic principle of the assay method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols of the crude extract. In addition aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A or B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorbance maximum at 510 nm. Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510 nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard. (Chang *et al.,* 2002)

Flavonoid (Extract) + $AlCl_3$ (reagent) = Formation of flavonoid-aluminium complex (λ_{max} = 510 nm)

3.6.2.2 Apparatus & Reagents

Table 3.4: Apparatus and reagents used for total flavonoid content

Aluminium chloride	Spatula
Methanol	Analytical balance
Quercetin	Pipette and pumper
Sodium hydroxide	Aqueous fraction
Sodium nitrite	Test tubes and beaker

3.6.2.3 Procedure

Preparation of 10% Aluminium Chloride ($AlCl_3$) Solution: 1gm of $AlCl_3$ was taken into a 10 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of 4% NaOH Solution: 4 gm of NaOH was taken into a 100 ml volumetric flask and the volume was adjusted by distilled water.

Preparation of 5% (W/V) NaNO2 Solution: 0.5 gm of $NaNO_2$ was taken into a 10 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of Standard Solution: The stock solution was prepared by taking 10 mg of quercetin and dissolved into 50 ml of methanol. Concentration of this solution was 200 µg/ml of quercetin. The experimental concentrations were prepared from this stock solution.

Table 3.5: Preparation of standard solution

Concentration (µg/ml)	Solution taken from stock solution (ml)	Solution taken from stock solution (ml)	Final volume (ml)
0	0.0	5.0	5
4	0.1	4.9	5
8	0.2	4.8	5
12	0.3	4.7	5
16	0.4	4.6	5

Preparation of Extract Solution: 5 mg of each plant extracts were taken and dissolved into 5 ml of methanol. The concentration of the solution was 1 mg/ml of plant extracts. Then the following steps were carried out.

1.5 ml extract was taken in a test tube and then 6 ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for

6minutes. 4% NaOH and 0.6 ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5 ml methanol was taken and same procedure was repeated.

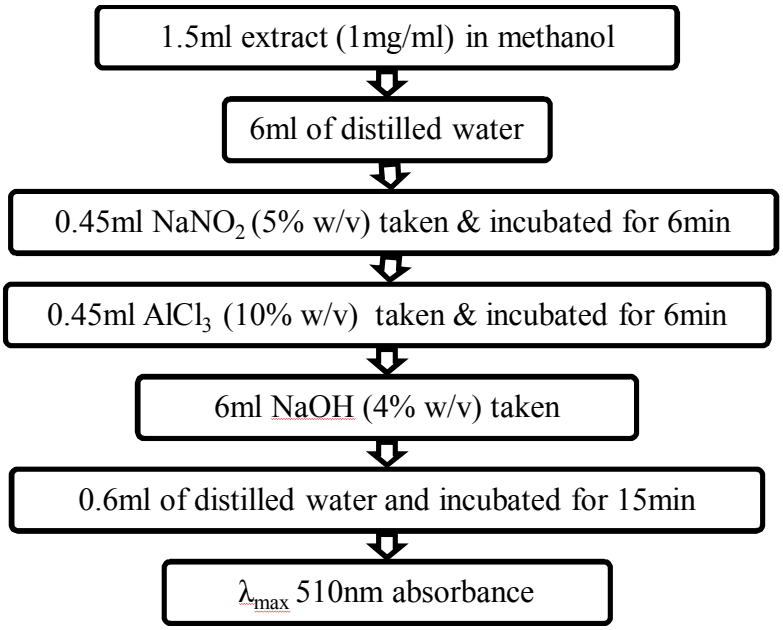


Figure 3.5: Schematic diagram of preparation of extract solution

Preparation of blank solution

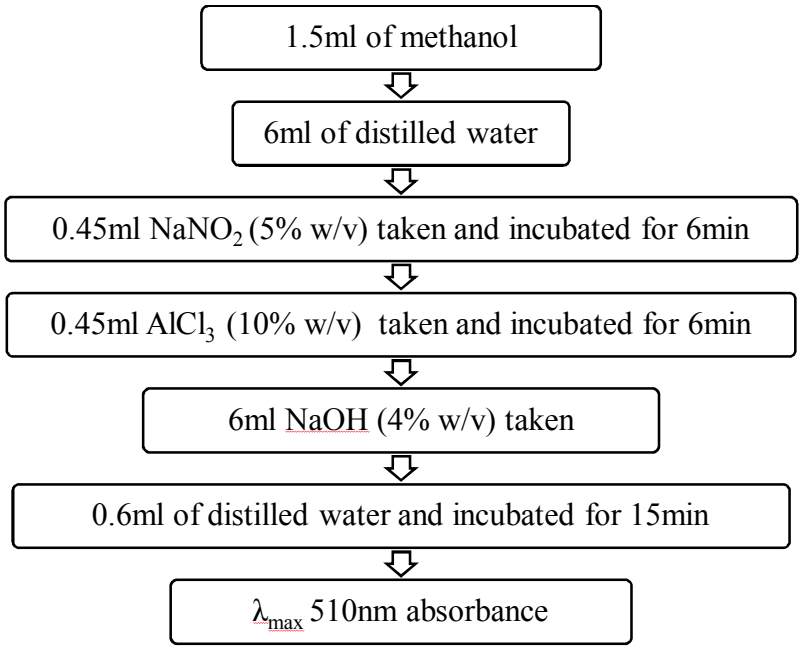


Figure 3.6: Schematic diagram of preparation of blank solution

3.7 Antimicrobial Activity by Disc Diffusion Method

3.7.1 Principle

The disk diffusion susceptibility method is simple and well-standardized. Bacterial inoculums are applied to the surface of a large agar plate. Antibiotic discs and disc of test materials are placed on the inoculated agar surface. Plates are incubated for 16–24hr at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The zones of growth inhibition are measured to the nearest millimeter around each of the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium (Barry, 1976).

3.7.2 Apparatus &Reagents

Table 3.6: Apparatus and reagents for antimicrobial test

Filter paper discs	Screw cap test tubes
Petri dishes	Nose mask and Hand gloves
Inoculating loop	Laminar air flow hood
Sterile cotton	Autoclave
Sterile forceps	Incubator
Spirit burner	Ethanol
Micropipette	Nutrient Agar Medium

3.7.3 Test Sample of *Syzygium samarangense*(leaves)

DCM fraction of methanolic extract of *Syzygium samarangense* leaves were taken as test sample.

3.7.4 Test Organisms

The bacterial strains used for the experiment were collected as pure cultures from the East West University microbiology laboratory. Both gram positive and gram-negative organismswere taken for the test and they are listed in the following table.

Table 3.7: List of micro-organisms

Type of Bacteria	Name of Bacteria
Gram positive(+ve)	*Bacillus sereus*
	Bacillus subtilis
	Bacillus megaterium
	Staphylococcus aureus
Gram negative (–ve)	*Escherichia coli*
	Salmonella typhi
	Salmonella paratyphi
	Vibrio parahaemolyticus
	Vibrio mimicus
	Shigella dysenteriae
Fungi	*Candida albicans*
	Aspergillus niger

3.7.5 Procedure

3.7.5.1 Preparation of the Medium

To prepare required volume of this medium, 56 gm of agar medium was taken in a bottle with a cap and distilled water was added to it to make 200ml volume. The contents were then autoclaved to make a clear solution.

Figure 3.7: Autoclave machine

3.7.5.2 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121º C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

3.7.5.3 Prep

The test org ut 10 ml of
melted and ı by a loop
mixed with ɔn bud was
taken and d ɔlied to the
petridish wit

3.7.5.4 Prep

Three types

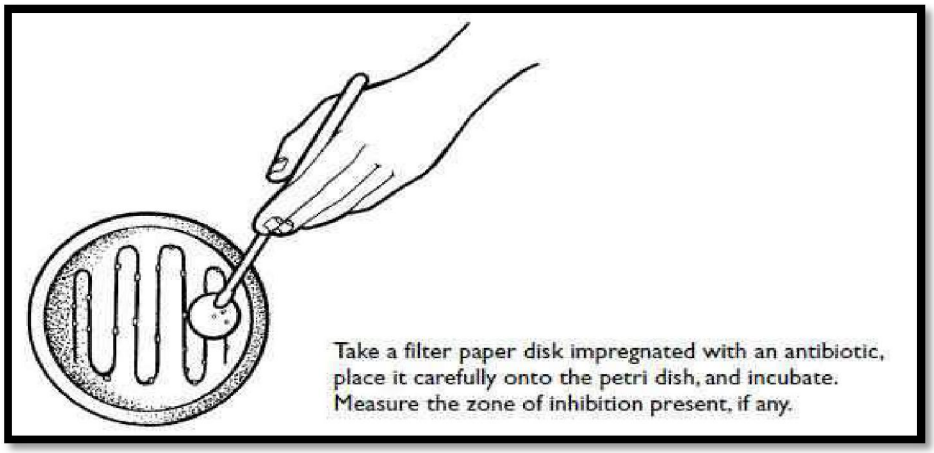


Figure 3.9: Preparation of filter paper discs

- **Standard Discs:** These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, ciprofloxacin (30µg/disc) disc was used as the reference.
- **Blank Discs:** These were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.
- **Sample Discs:** These discs were soaked with solutions of test samples of known concentration, dried and used to determine the anti-activity of the samples.

3.7.5.5 Preparation of Test Sample

Measured amount of test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical filter paper discs were taken in a blank petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

3.7.5.6 Application of Test Samples

Standard ciprofloxain discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Methanol discs were used as negative controls which ensure that the residual solvents (left over the discs even after airdrying) and the filter paper were not active themselves.

3.7.5.7 Diffusion & Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours.

Figure 3.10: Incubator

3.6.5.8 Determination of Antimicrobial Activity by Measuring the Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

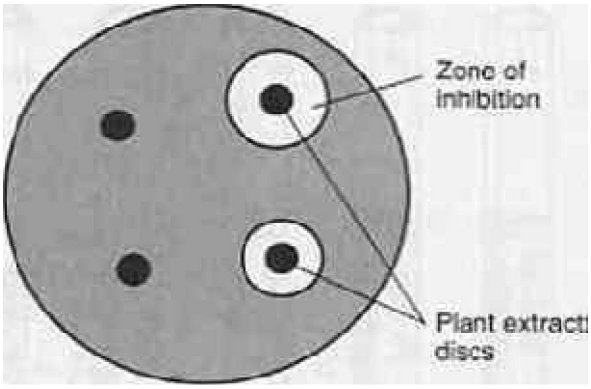


Figure 3.11: Clear zone of inhibition

Figure 3.12: Determination of clear zone of inhibition

Chapter Four
Results and Discussion

Results and Discussion

4.1 Antioxidant test results

Antioxidant tests are classified by various methods. Samples were subjected to various standard methods to determine various scavenging capacity and amount that is equivalent to the standard like ascorbic acids. Antioxidant property of the Dichloromethane (DCM) fraction of methanolic extract of *S. samarangense* (leaves) was determined by following methods-

- Determination of total phenolic content
- Determination of total flavonoids content

4.1.1. Result of Total Phenolic Content

The Dichloromethane extract of leaves of *S. samarangense* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard. (Singleton *et al.*, 1999).

4.1.1.1. Preparation of Standard Curve

Table 4.1: Total phenol content of ascorbic acid

Concentration (µg/ml)	Absorbance (at 765 nm)	Regression line	R^2 value
80	2.406	y = 0.0193x + 0.8246	0.9372
90	2.473		
100	2.767		
110	3.057		
120	3.080		

A linear relationship was observed when the absorbances were plotted against concentrations, asshown in Figure 4.1. This linear curve was considered as a standard curve.

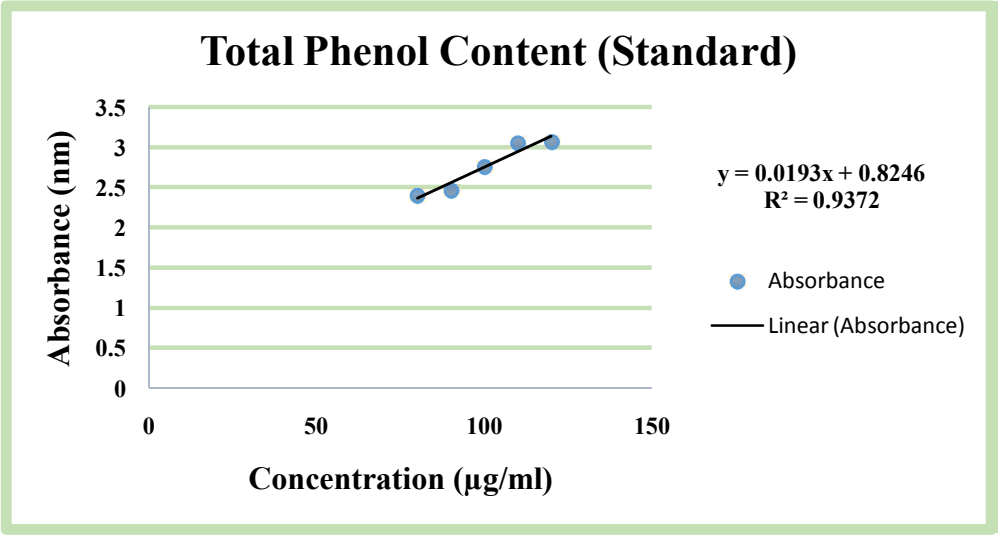


Figure 4.1: Graphical Representation of assay of phenolic content of Ascorbic Acid.

4.1.1.2. Total Phenol Content Present in DCM Extract of *S. samarangense*

Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent andcompared with the standard solutions of ascorbic acid equivalents (AAE), the total phenolic content present in the extract is calculated and given in the table below.

Table 4.2. Total Phenolic content in DCM fraction of *S. samarangense* (leaves)

Sample	Concentration (mg/ml)	Absorbance (Y value at 765 nm)	Total Phenolic (X) value (mg of AAE/gm of dried extract)
DCM fraction of *S. samarangense*	2	1.638	21.073

4.1.1.3. Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the DCM fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 21.073 mg of AAE/gm of dried extract of phenol content was found in the DCM fraction of *S. samarangense.*

4.1.2. Result of Total Flavonoid Content

The DCM fractions of *S. samarangense*(leaves)were subjected to determine total flavonoid content.Quercetin was used as reference standard.

4.1.2.1. Preparation of Standard Curve

Table 4.3. Total Flavonoid content of Quercetin

Concentration (µg/ml)	Absorbance (at 510 nm)	Regression line	R^2 value
4	0.193	y = 0.053x - 0.013	0.9992
8	0.422		
12	0.618		
16	0.834		

After absorbances were taken of different solution of quercetin of concentrations ranging from 4 µg/mlto 16µg/ml, a linear relationship was observed when the absorbance were plotted against concentrations, as shown in Figure 4.2. This linear curve was considered as a standard curve.

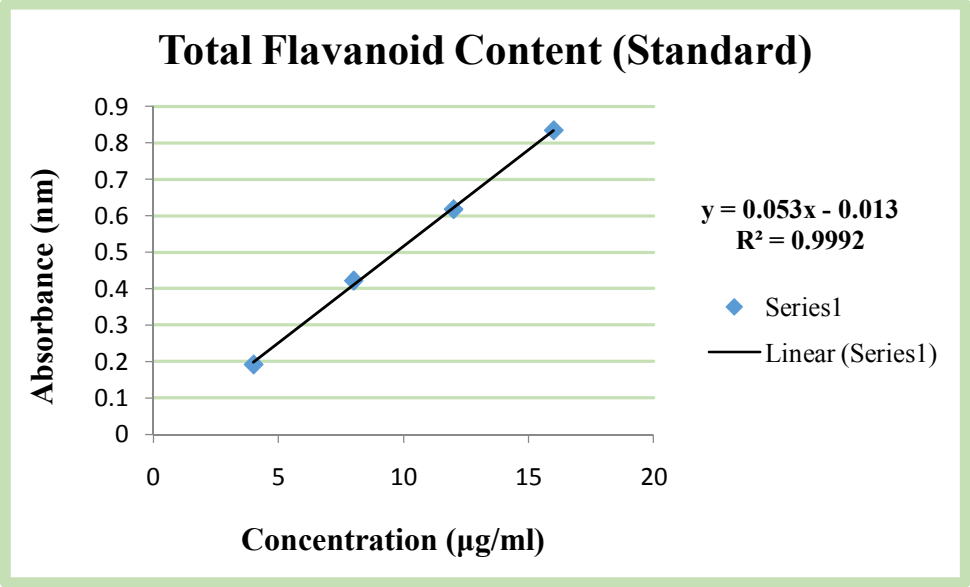


Figure 4.2: Graphical representation of Flavonoid content of quercetin.

4.1.2.2. Total Flavonoid Content Present in DCM fraction of *S. samarangense* (leaves)

Based on the absorbance value of extract solution and using the regression line equation of the standard curve, the total flavonoid present in the extract is calculated and is given in Table 4.4.

Table 4.4. Total Flavanoid content in DCM fraction of *S. samarangense* (leaves)

Sample	Concentration (mg/ml)	Absorbance (Y value at 510 nm)	Total Flavanoid (X) value (mg of quercetin/gm of dried extract)
DCM fraction of *S. samarangense*	5	0.141	0.581

4.1.2.3. Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increasedwith the increase in concentration indicating increase in flavonoid content. Absorbance of the DCM fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 0.581 mg of Quercetin/gm of dried extract of flavonoid content was found in the DCM fraction of *S. samarangense* (leaves).

4.2. Result of Antimicrobial Test

The antimicrobial activities of DCM fraction of *S. samarangense* leaves extract were subjected in the study against various Gram positive bacteria, Gram negative bacteria and fungi. The DCM fraction was subjected to the various bacterial and fungal cultures and from that zones of inhibition were measured. Ciprofloxacin was used as standard reference.

4.2.1. Zone of Inhibition of Standard and DCM Fraction

Table 4.5. Antimicrobial activity of standard sample (Ciprofloxacin) and DCM fraction

Type of microorganism		Zone of inhibition (mm)	
		Standard sample	DCM fraction
Gram positive bacteria	*Bacillus sereus*	31	7
	Bacillus subtilis	31	7
	Bacillus megaterium	30	5
	Staphylococcus aureus	31	8
Gram negative bacteria	*Escherichia coli*	30	7
	Salmonella typhi	30	9
	Salmonella paratyphi	32	6
	Vibrio parahaemolyticus	30	10

	Vibrio mimicus	30	8
	Shigella dysenteriae	33	6
Fungi	*Candida albicans*	30	7
	Aspergillus niger	30	7

4.2.2. Discussion

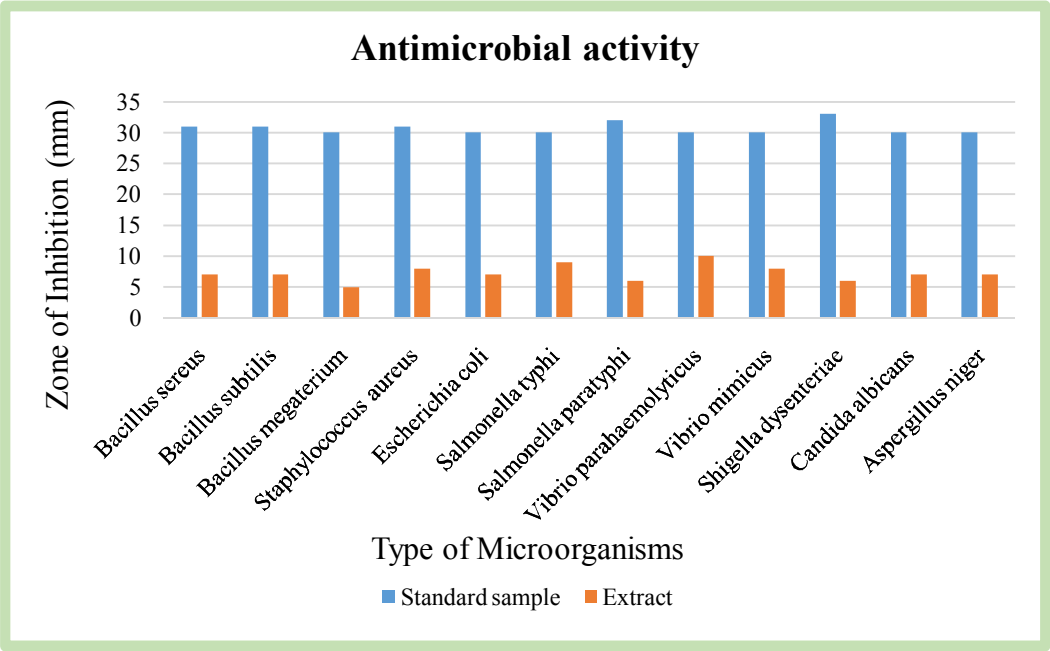


Figure 4.3. Comparison of antimicrobial activity between standard and extract

DCM fraction of *S. samarangense* leaves extract showed low to moderate antimicrobial activity when compared to reference standard drug Ciprofloxacin. None of the zone of inhibition of DCM fraction is equal to Ciprofloxacin against any bacteria or fungi as shown in the Figure: 4.3. Among all the microbiological cultures, the fraction showed the best antimicrobial activity against *Vibrio parahaemolyticus*(10 mm) comparable to the standard (30 mm).

4.3. Result of Brine Shrimp Lethality Bio-Assay

The DCM fraction of the *S. samarangense* (leaves) extract was subjected to brine shrimp lethality bioassay. After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a Median Lethal Concentration (LC_{50}) value. LC_{50} represents the concentration of the standard and DCM extract that produces death in half of the test subjects after a certain period. The percentage mortality at each concentration was determined using the following formula.

$$\%\,\text{Mortality} = \frac{\text{Number of dead nauplii} \times 100}{\text{Total number of nauplii}}$$

The LC_{50} of the test samples was obtained by a plot of percentage of the shrimps died (% Mortality) against the logarithm of the sample concentration (Log C) and the best-fit line was obtained from the curve data by means of regression analysis.

4.3.1. Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard.

Table 4.6. Results of the bioassay of Tamoxifen (standard)

Test tube number	Concentration (C) (µg */ml*)	Log C	Number of alive nauplii	Number of dead nauplii	% Mortality	LC_{50} (µg */ml*)
1	400	2.602	0	10	100	
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	3	7	70	
5	25	1.398	5	5	50	13.38
6	12.5	1.097	5	5	50	
7	6.25	0.796	6	4	40	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	0.78125	-0.107	9	1	10	

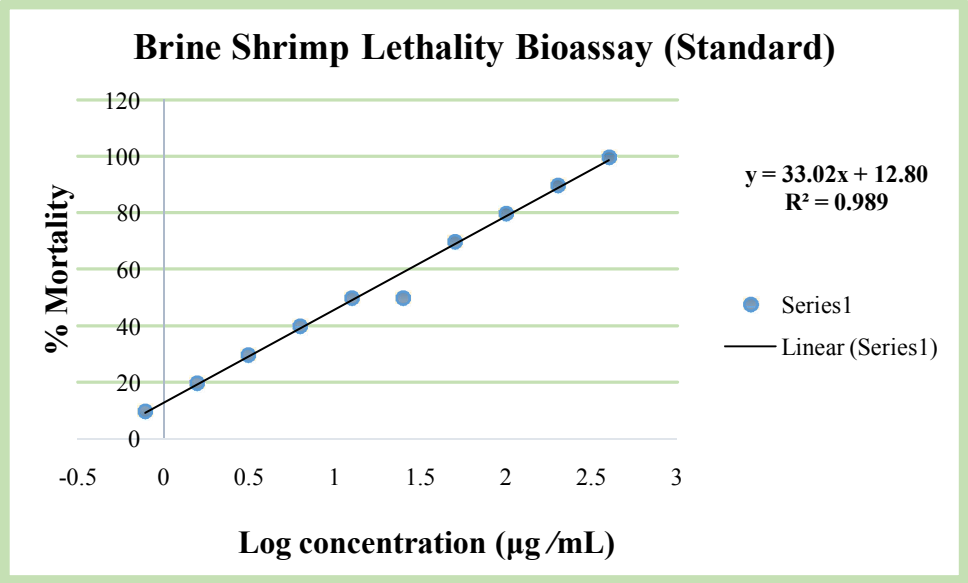


Figure 4.4. % Mortality and Predicted Regression Line of Tamoxifen (standard)

4.3.2. Preparation of DCM Fraction Curve of *S. samarangense* (leaves)

Table 4.7. Results of the bioassay in DCM fraction of *S. samarangense* (leaves)

Test tube number	Concentration (C) (µg /*ml*)	Log C	Number of alive nauplii	Number of dead nauplii	% Mortality	LC_{50} (µg /*ml*)
1	400	2.602	0	10	100	
2	200	2.301	0	10	100	
3	100	2.000	1	9	90	
4	50	1.699	1	9	80	
5	25	1.398	2	8	70	2
6	12.5	1.097	2	8	80	
7	6.25	0.796	3	7	70	
8	3.125	0.495	4	6	60	
9	1.5625	0.194	6	4	40	
10	0.78125	-0.107	7	3	30	

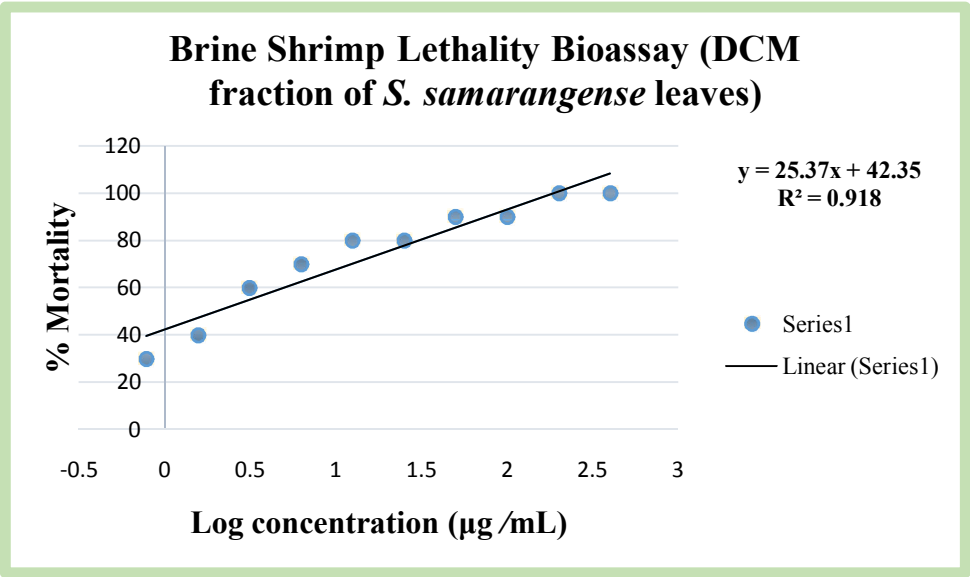


Figure 4.5. % Mortality and Predicted Regression Line in DCM fraction of *S. samarangense* (leaves)

4.3.3Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure todifferent concentrations of the test samples. The degree of lethality was found to be directlyproportional to the concentration. Maximum mortalities took place at the concentrationof 400 and 200 µg/ml, whereas the least mortalities at the concentration of 0.78125µg/ml as shown inTable 4.7.

Table 4.8. Cytotoxic activity of Tamoxifen and DCM fraction of *S. samarangense* (leaves).

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml)
Standard (Tamoxifen)	y = 33.021x + 12.806	0.9891	13.38
DCM fraction	y = 25.37x + 42.351	0.9181	2

In this investigation, standard and DCM fraction exhibited cytotoxic activities with the LC_{50} values at13.38 µg/ml and 2 µg/ml respectively as shown in Table 4.8. LC_{50} value of

Sygyziumsamarangense (leaves)in DCM fraction showed more activity of it than Tamoxifen. Further investigation is needed to confirm the activity.

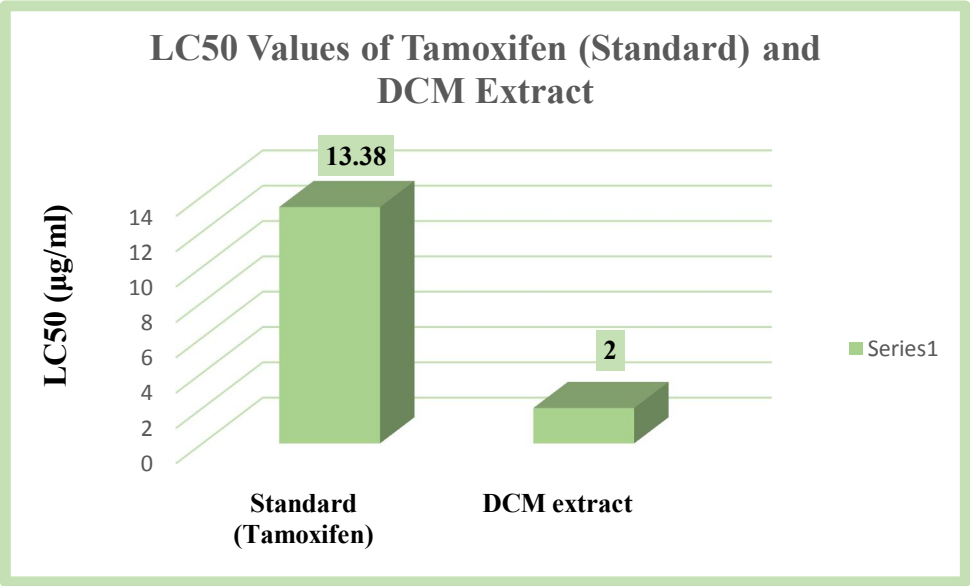


Figure 4.6. Comparison between LC_{50} values of standard and extract

Chapter Five
Conclusion

Conclusion

As the literature review suggests, the presence of several phytochemical compounds in DCM fraction of *S. samarangense* (leaves), makes the plant pharmacologically active.

LC_{50} value of *S. samarangense* (leaves)in DCM fraction showed more cytotoxic activity than Tamoxifen. Since DCM fraction of *S. samarangense* (leaves) exhibited potent cytotoxic activity, so it can be investigated for anticancer, pesticidal and antitumor properties in future.

Antioxidant property in DCM extract of *S. samarangense* (leaves) was determined by Phenolic content assay and Flavonoid content assay. Phenolic content was 21.073 mg/gm and Flavonoid content was 0.581 mg/gm in DCM extract of *S. samarangense (leaves)*. So,DCM extract of *S. samarangense* (leaves) have poor antioxidant property. Mixture of compounds can lower antioxidant property in DCM fraction of *S. samarangense* (leaves), if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of DCM fraction of *S. samarangense* (leaves)

The study also showed that, the extract showed low to moderate antimicrobial activity that could be a better treatment in antimicrobial infections.

However, studies are required on higher animal model and subsequently on human subjects to prove efficacy as an antioxidant,cytotoxic and antimicrobial agent. It will help in the development of new novel and safe drugs for the treatment of various diseases.

Chapter Six
Reference

Reference

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