Cytotoxic, antimicrobial & antioxidant activity of Ethyl Acetate Fraction of *Syzygium Samarangense* leaves 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.

Submitted By

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DECLARATION BY THE CANDIDATE

I, Humayra Halim Antora, hereby declare that this dissertation, entitled "Cytotoxic, antimicrobial & antioxidant activity of Ethyl Acetate Fraction of *Syzygium Samarangense* leaves extract" 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. 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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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled "Cytotoxic, antimicrobial & antioxidant activity of Ethyl Acetate Fraction of *Syzygium Samarangense* leaves extract" is a research work done, under our guidance and supervision by Humayra Halim Antora (ID: 2011-3-70-046) 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, they are my biggest inspirations.

Abstract

The plant *Syzygium Samarangense* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine. The aim of the present study was to evaluate the cytotoxicity activity, antimicrobial activity and antioxidant activity of ethyl acetate extract of *Syzygium Samarangense*. The powdered leaves of *Syzygium Samarangense* were extracted with methanol and then partitioned with n-hexane, dichloromethane and ethyl acetate consecutively. The ethyl acetate fraction was used to evaluate cytotoxic, antimicrobial and antioxidant activities. The cytoxic activity was measured by brine shrimp lethality bioassay. Ethyl acetate fraction showed cytotoxic activity with LC50 value 3.45µg/ml in brine shrimp lethality test. The antimicrobial activities of ethyl acetate solvent extract of *Syzygium Samarangense* plant were tested against the Gram-positive and Gram-negative bacterial strains by observing the zone of inhibition. The antimicrobial test was performed by Disc diffusion method. The crude ethyl acetate extract of *Syzygium Samarangense*. The fraction contained 160.48mgAAE/g of total phenolic content and 1.302mg AAE/g total flavonoid content. The results of study indicate the presence of cytotoxic, antimicrobial and antioxidant properties of ethyl acetate extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation

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

LIST OF CONTENTS

List of Figures:

List of Tables:

Abbreviations:

Meaning of abbreviated form	Abbreviated form
Ascorbic Acid Equivalent	AAE
Dimethyl Sulfoxide	DMSO
Gram	g or gm
Milligram	mg
Microgram	µg
Milliliter	ml
Micro liter	µl
Hour	hr
Lethal concentration required to kill 50% of the sample population	LC_{50}
Opuntia	O.
Ultraviolet	UV
World Health Organization	WHO
Syzygium Samarangense	*S. samarangense*

CHAPTER ONE

INTRODUCTION

1.1 Eukaryote

The primary taxa of eukaryote classification should be monophyletic and based on fundamental cell structure rather than nutritional adaptive zones. The classical two kingdom classification into "plants" and "animals" and the newer four kingdom classifications into "protist", "fungi", "animals" and "plants" are therefore both unsatisfactory. Eukaryotes can be classified into nine kingdoms each defined in terms of a unique constellation of cell structures.

Five kingdoms (1 to 5) have plate-like mitochondrial cristae:

i. Kingdom 1: Eufungi (the non-ciliated fungi, which unlike the other eight kingdoms have unstacked Golgi cisternae),

ii. Kingdom 2: Ciliofungi (the posteriorly ciliated fungi),

iii. Kingdom 3: Animalia (Animals, sponges, mesozoa, and choanociliates; phagotrophs with basically posterior ciliation),

iv. Kingdom 4: Biliphyta (Non-phagotrophic, phycobilisome-containing, algae; i.e. the Glaucophyceae and Rhodophyceae),

v. Kingdom 5: Viridiplantae (Non-phagotrophic green plants, with starch-containing plastids).

vi. Kingdom 6: the Euglenozoa, has disc-shaped cristae and an intraciliary dense rod and may be phagotrophic and/or phototrophic with plastids with three-membraned envelopes.

vii. Kingdom 7: The Cryptophyta, has flattened tubular cristae, tubular mastigonemes on both cilia, and starch in the compartment between the plastid endoplasmic reticulum and the plastid envelope; their plastids, if present, have phycobilins inside the paired thylakoids and chlorophyll c_2.

viii. Kingdom 8: The Chromophyta, has tubular cristae, together with tubular mastigonemes on one anterior cilium and/or a plastid endoplasmic reticulum and chlorophyll $c_2 + c_2$.

ix. Kingdom 9: The Protozoa, are mainly phagotrophic, and have tubular or vesicular cristae (or lack mitochondria altogether), and lack tubular mastigonemes on their (primitively

anterior) cilia; plastids if present have three-envelope membranes, chlorophyll c_2, and no internal starch, and a plastid endoplasmic reticulum is absent. Kingdoms 4–9 are primitively anteriorly biciliate.

Detailed definitions of the new kingdoms and lists of the phyla comprising them are given. Advantages of the new system and its main phylogenetic implications are discussed. A simpler system of five kingdoms suitable for very elementary teaching is possible by grouping the photosynthetic and fungal kingdoms in pairs. Various compromises are possible between the nine and five kingdom systems; it is suggested that the best one for general scientific use is a system of seven kingdoms in which the Eufungi and Ciliofungi become subkingdoms of the Kingdom Fungi, and the Cryptophyta and Chromophyta subkingdoms of the Kingdom Chromista; the Fungi, Viridiplantae, Biliphyta, and Chromista can be subject to the Botanical Code of Nomenclature, while the Zoological Code can govern the Kingdoms Animalia, Protozoa and Euglenozoa. If one accepts the idea that the Ciliofungi evolved directly from a eufungus (or vice versa) and that the Chromophyta evolved from a cryptophyte, then these seven kingdoms would probably be monophyletic and preferable to the nine-kingdom system, which is my own view. The nine-kingdom system is independent of these particular phylogenetic assumptions and may therefore be preferred by those who reject them. (Cavaliar Smith, T., 1981)

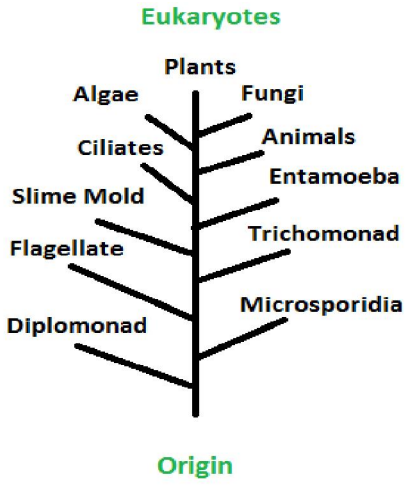


Figure1.1: Eukaryotes

1.2 Plant

Plant, any member of the kingdom Plantae, multicellular eukaryotic life forms characterized by

- Photosynthetic nutrition(a characteristic possessed by all plants except some parasitic plants and underground orchids), in which chemical energy is produced from water, minerals, and carbon dioxide with the aid of pigments and the radiant energy of the Sun.
- Essentially unlimited growth at localized regions.
- Cells that contain cellulose in their walls and are therefore to some extent rigid.
- The absence of organs of locomotion, resulting in a more or less stationary existence.
- The absence of nervous systems.
- Life (Schmid et al., 2016)

Figure1.2: Plant

1.2.1 Medicinal Plants

The plants which are useful for healing several diseases are called medicinal plant. A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness. Typically, pharmaceutical drugs are modeled after compounds found in medicinal plants. (Peterson Munks, B 2016)

Figure1.3: Medicinal Plants

Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly 50,000 species of higher plants (about 1 in 6 of all species) have been used medicinally. This represents by far the biggest use of the natural world in terms of number of species.

Figure1.4: Medicinal Plants

Most species are used only in folk medicine, traditional systems of formal medicine using relatively few (e.g. 500-600 commonly in Traditionally Chinese Medicine). Around 100 plant species have contributed significantly to modern drugs. The use of medicinal plants is increasing worldwide, related to the persistence and sometimes expansion of traditional medicine and a growing interest in herbal treatments. The medicinal uses of plants grade into their uses for other purposes, as for food, cleaning, personal care and perfumery. Plants are used in medicine to

maintain and augment health - physically, mentally and spiritually - as well as to treat specific conditions and ailments. (plant life international ,2016)

1.2.2 Herb

Simple Definition of herb is a plant or a part of a plant that is used as medicine or to give flavor to food. The word "**herb**" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now a day, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. (Alam Khan, D., 2016)

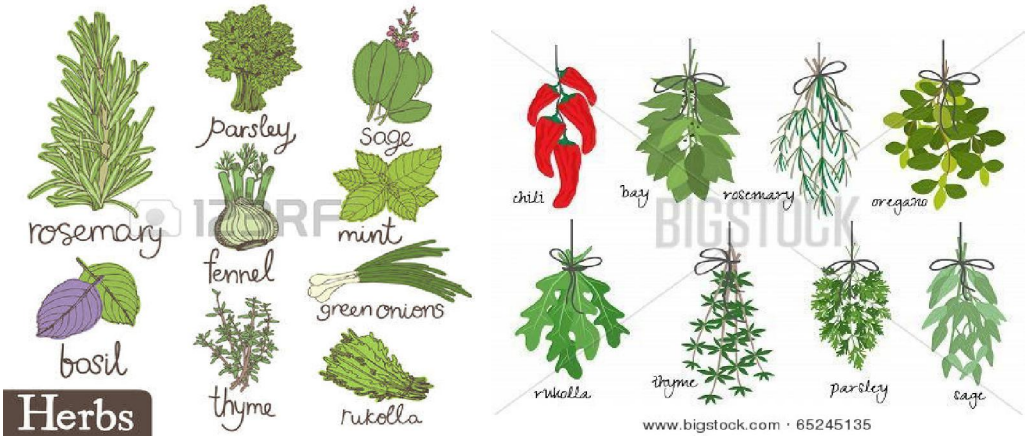


Figure1.5: Herb

The dictionary gives two definitions for "herb." The first one, the more technical, is "a seed producing plant that does not develop persistent woody tissue but dies down at the end of the growing season." The second definition is more general, the one most commonly used, and the one that I am referring to when I use the word. An herb is "a plant or plant part valued for its medicinal, savory, or aromatic qualities."

A "plant or plant part" means that an herb can be a single-celled algae, the bark of a tree, the leaf, root, fruit, seed, flower, or any other part of any plant, as long as it is used for its medicinal, savory, or aromatic qualities.

"Medicinal" means for health purposes, including the treatment and prevention of disease. "Savory" means herbs that are used to season food, such as garlic, rosemary, basil, etc. And "aromatic" refers to herbs, plant parts, or flowers that are used for their odor or aroma, as in perfumes, fragrances, incense, etc. There are numerous other uses for herbs that do not easily fit into these three categories. For example, herbs are used in the preservation of food, for dying cloth, pest control, decorating, and for many other purposes. Did I forget to mention that herbs are often used for food?

"Let your food be your medicine, and let your medicine be your food." —Hippocrates, the father of Western medicine. (Introduction to Herbalism, 2016)

1.3 History of Medicine

The History of Medicine - humans have been practicing medicine in one way or another for over a million years. In order to understand how modern medicine got to where it is now, it is important to read about the history of medicine. In this series of articles, you can read about:

- Prehistoric Medicine
- Ancient Egyptian Medicine
- Ancient Greek Medicine
- Ancient Roman Medicine
- Medieval Islamic Medicine
- Medieval and Renaissance European Medicine
- Medicine from the 18th century until today (Nordqvist, C., 2012)

1.3.1 Prehistoric medicine

Prehistoric medicine refers to medicine before humans were to read and write. It covers a vast period, which varies according to regions and cultures. Anthropologists, people who study the history of humanity, can only make calculated guesses at what prehistoric medicine was like by collecting and studying human remains and artifacts. They have sometimes extrapolated from

observations of certain indigenous populations today and over the last hundred years whose lives have been isolated from other cultures.

People in prehistoric times would have believed in a combination of natural and supernatural causes and treatments for conditions and diseases. The practice of comparing a placebo effect with a given therapy did not exist. There may have been some trial and error in coming to some effective treatments, but they would not have taken into account several variables scientists factor in today, such as coincidence, lifestyle, family history, and the placebo effect. Nobody can be absolutely certain what prehistoric peoples knew about how the human body works.

1.3.2 The Ancient Egyptians

The **Ancient Egyptians**, like the Ancient Greeks and Romans, have provided modern historians with a great deal of knowledge and evidence about their attitude towards medicine and the medical knowledge that they had. This evidence has come from the numerous papyruses found in archaeological searches.

Like prehistoric man, some of the beliefs of the Egyptians were based on myths and legend. However, their knowledge was also based on an increasing knowledge of the human anatomy and plain commonsense.

In Ancient Egypt, the treatment of illnesses was no longer carried out only by magicians and medicine men. We have evidence that people existed who were referred to physicians and doctors. (Trueman, C., 2015)

1.3.3 The Ancient Greece

Ancient Greece, as with Ancient Rome and Ancient Egypt, played an important part in medical history. The most famous of all Ancient Greek doctors was Hippocrates. By 1200 B.C., Ancient Greece was developing in all areas – trade, farming, warfare, sailing, craftsmanship etc. Their knowledge of medicine developed accordingly.

Gods dominated the lives of the Greeks. Natural occurrences were explained away by using gods. This, however, did not occur in medicine where Ancient Greek physicians tried to find a natural explanation as to why someone got ill and died.

The Greeks were practicing medicine 1000 years before the birth of Christ. In the 'Iliad' by Homer, injured soldiers were treated by doctors and the Greek leader in the tale, Menelaus, was treated for an arrow wound by a doctor-in-arms, Machaon. (Trueman, C., 2015)

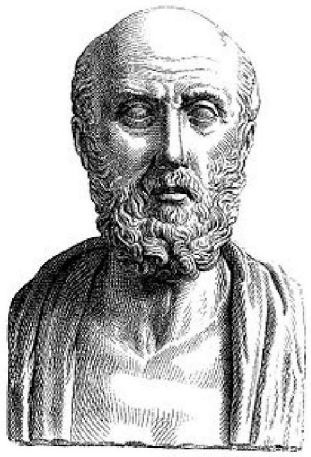


Figure1.6: Hippocrates

Hippocrates and other Greek doctors believed that the work done by a doctor should be kept separate from the work done by a priest. They believed that observation of a patient was a vital aspect of medical care. Ancient Greek doctors did examine their patients but Hippocrates wanted a more systematic period of observation and the recording of what was observed. Today, we would call this 'clinical observation'. Such ideas have lead to **Hippocrates** being called the **'Father of Medicine'. (**Trueman, C., (2015)

1.3.4 The Ancient Romans

The Ancient Romans, like the Ancient Greeks and Ancient Egyptians, made a huge input into medicine and health, though their input was mainly concerned with public health schemes. Though the Roman 'discoveries' may not have been in the field of pure medicine, poor hygiene by people was a constant source of disease, so any improvement in public health was to have a major impact on society.

The Romans learned a great deal from the Ancient Greeks. They first came into contact with the Greeks in about 500 BC By 146 B.C. part of Greece had become a province of the Roman Empire and by 27 B.C., the Romans were in control not only of Greece but of Greek-speaking lands around the Mediterranean. They used the ideas of the Greeks but they did not simply copy them. Greek ideas they found impractical they ignored and it seems that the Romans were more keen on things that would lead to the direct improvement of the quality of life of the people in their huge empire. (Trueman C T., 2015)

1.3.5 Medicine was an important part of medieval Islamic life

Islamic medicine was not a subject in its own right in the medieval Islamic world - it was immersed into the culture. The publication of literature was a prestigious profession - paper making was sophisticated, books had illustrations, calligraphy was considered an art in itself. For readers of medicine at the time, those published in the Islamic world were fascinating to read. From 661 to 750 AD, an early Islamic period called Umayyad; people generally believed that Allah (God) would provide treatment for every illness. By 900 AD Islam started to develop and practice a medical system slanting towards science.As people became more interested in health and health sciences from a scientific point of view, Islamic doctors strived to find healing procedures, with Allah's permission, that looked at the natural causes and potential treatments and cures.

The Medieval Islamic world produced some of the greatest medical thinkers in history; they also made advances in surgery, built hospitals, and welcomed women into the medical profession. (Nordqvist, C., 2016)

1.3.6 Medieval and Renaissance European Medicine

The **Medieval Period**, commonly known as **The Middle Ages** spanned 1,000 years, from the 5th to the 15th century (476 AD to 1453 AD). It is the period in European history which started at the end of Classical Antiquity (Ancient History), about the time of the fall of the Western Roman Empire, until the birth of the Renaissance period and the Age of Discovery. The Middle Ages is divided into three periods - the Early, High and Late Middle Ages. The Early Middle Ages are also known as the Dark Ages. Many historians, especially Renaissance scholars, viewed the Middle Ages as a period of stagnation, sandwiched between the magnificent Ancient

Roman period and the glorious Renaissance. The Renaissance period (1400s to 1700s) followed the Middle Ages. (Nordqvist, C., 2016)

1.3.7 Medicine from the 18th century until today

Modern medicine includes many fields of science and practice, including:

- **Clinical practice** - the physician assesses the patient personally; the aim being to diagnose, treat, and prevent disease using his/her training and clinical judgment.
- **Healthcare science** - a multidisciplinary field which deals with the application of science, technology, engineering (mathematics) for the delivery of care. A healthcare scientist is involved with the delivery of diagnosis, treatment, care and support of patients in systems of healthcare, as opposed to people in academic research. A healthcare scientist actively combines the organizational, psychosocial, biomedical, and societal aspects of health, disease and healthcare.
- **Biomedical research** - a broad area of science that seeks ways to prevent and treat diseases that make people and/or animals ill or causes death. It includes several areas of both physical and life sciences. Biomedical scientists use biotechnology techniques to study biological processes and diseases; their ultimate objective is to develop successful treatments and cures. Biomedical research requires careful experimentation, development and evaluations involving many scientists, including biologists, chemists, doctors, pharmacologist, and others. It is an evolutionary process.
- **Medications** - drugs or medicines and their administration. Medications are chemical substances meant for use in medical diagnosis, treatment, cure, or prevention of disease.
- **Surgery** - a branch of medicine that focuses on diagnosing and treating disease, deformity and injury by instrumental and manual means. This may involve a surgical procedure, such as one that involves removing or replacing diseased tissue or organs. Surgery usually takes place in a laboratory, operating room (theater), a dental clinic, or a veterinary clinic/practice.

- **Medical devices** - instruments, implants, in vitro reagents, apparatuses, or other similar articles which help in the diagnosis of diseases and other conditions. Medical devices are also used to cure disease, mitigate harm or symptoms, to treat illness or conditions, and to prevent diseases. They may also be used to affect the structure or function of parts of the body. Unlike medications, medical devices achieve their principal purpose (action) by mechanical, thermal, physical, physic-chemical, or chemical means. Medical devices range from simple medical thermometers to enormous, sophisticated and expensive image scanning machines.

- **Alternative medicine** - includes any practice which claims to heal but does not fall within the realm of conventional/traditional medicine. In most cases, because it is based on cultural or historical traditions, instead of scientific evidence. Scientific refers to, for example, demonstrating the effectiveness or a therapy or drug in a double-blind, random, long-term, large clinical human study (clinical trial), in which the therapy or drug is compared to either a placebo or another therapy/drug. Examples of alternative medicine include homeopathy, acupuncture, ayurveda, naturopathic medicine, and traditional Chinese medicine.

- Psychotherapy, physical therapy (UK: physiotherapy), occupational therapy, nursing, midwifery, and several other fields. (Nordqvist, C., 2012)

1.4 Necessity of Drug Development from Plant Sources

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role. Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents. So the application of herbal drug in crude form may be ineffective or may cause a toxic reaction.

- Vincristine, a prominent anticancer drug, was developed from periwinkle plant (Vinca rosea) which was formerly prescribed for treating diabetes. The efficient hypotensive

drug, reserpine, was developed from Rauwolfia serpentine which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN et al., 1982).

- Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from Ammi visnaga which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Ghani, 1998).

1.4.1 Procedure for Development

Sine drug development is an expensive practice, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required. The way of developing drugs from plants involves several stages (Ghani, 1998), which include:

- Selection and correct identification of the proper medicinal plant.
- Extraction with suitable solvent(s).
- Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.
- Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.
- Repeated fractionation of active fractions to isolate pure compound(s).
- Elucidation of chemical structure of pure compound(s) using spectroscopic methods.
- Evaluation of biological activity of pure compound(s)
- Toxicological tests with pure compound(s).
- Production of drug in appropriate dosage forms.

1.5 Importance of some herbs with their medicinal values

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.

- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be

planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.

- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.
- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.
- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as *Chirayta*, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.
- Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.
- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.
- Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.
- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.
- Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.
- Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.

- Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.

- Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.

- Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.

- Certain aromatic plants such as Aloe, Golden seal, Barberry and Chirayata are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.

- Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne (Lal Mirch, Myrrh, Camphor and Guggul.

- A wide variety of herbs including Giloe, Golden seal, Aloe and Barberry are used as tonics. They can also be nutritive and rejuvenate a healthy as well as diseased individual.

- Honey, turmeric, marshmallow and liquorice can effectively treat a fresh cut and wound. They are termed as vulnerary herbs. (Alam Khan, D., 2016)

1.6 Sources of medicine

Medicinal properties derived from plants can come from many different parts of a plant including leaves, roots, bark, fruit, seeds, flowers. The different parts of plants can contain different active ingredients within one plant. Thus, one part of the plant could be toxic while another portion of the same plant could be harmless.

Figure 1.7: Parts of Medicinal Plants

Medicinal properties can be derived from the following:

- **Bark:** The protective outer layer of a tree trunk that is formed by layers of living cells above the wood. Active ingredients are often found in higher concentrations in the bark. Examples of bark used for medicinal properties are quinine bark, oak bark, pepper bark, and willow bark.

- **Bulb:** A bulb is defined as a fleshy structure comprised of numerous layers of leaf bases otherwise known as bulb scales. Onion species and garlic bulbs are popular for medicinal uses.

- **Essential Oil:** These are defined as volatile oils that are generally extracted from plants using a steam distillation process. Examples include camphor and peppermint oil.

- **Fatty Oil:** These are defined as non-volatile vegetable oils that are pressed from the seeds or fruits of plants and are insoluble in water. Examples of fatty oils used in medicine are castor oil, olive oil, and safflower oil. Some fatty oils have direct medicinal properties while others are used as carriers in liquid formations and ointments.

- **Flowers:** The flowers of plants have always been popular in traditional medicine. Examples include clove and chamomile flowers. Flower parts are also used such as saffron stamens, the stigmas of maize, or pollen.

- **Fruit:** Fruits have been heavily used for medicinal purposes. Dried whole fruits or portions of fruits can be used. Many members of the carrot family have fruits that are used in medicine including fennel fruit and anise.

- **Gum:** Gums are solids that are mixtures of polysaccharides (sugars). They are water-soluble and are in part digestible by humans.

- **Leaf:** The leaves of plants, shrubs, and trees can be used for medicinal properties. Leaves can be used alone or can be mixed with twigs, stems, and buds. Examples include maidenhair tree.

- **Resins:** Resins are a mixture of essential oils and terpenes that are usually not soluble in water. They are excreted by specialized cells or in ducts of plants. Examples include frankincense, myrrh, and mastic.

- **Roots:** The fleshy or woody roots are used for medicinal purposes. Roots may be solid (ginseng), fibrous (stinging nettle), or fleshy (devil's claw).

- **Rhizome:** A rhizome is defined as a fleshy or woody elongated stem that usually grows horizontally below the ground. Rhizomes often produce leaves above the ground and roots into the ground. Several medicinal plants are used primarily for their rhizomes including: ginger, wild columbine, and bloodroot.

- **Seed:** The seeds of many plants are used for their medicinal properties. Seeds may be contained within a fruit or are sometimes used on their own. Juniper berries look like fruits but they are actually seeds surrounded by beautiful woody cones.

- **Tuber:** A tuber is defined as a swollen, fleshy structure below ground. Tubers are usually of stem origin but can be partly stem and root in origin. Tubers used for medicinal properties include African potato and autumn crocus.

- **Wood:** Thick stems or the wood of trees or shrubs are used for medicinal properties. Sandalwood and quassia wood are popular examples. (Medicinal Botany., 2016)

1.7 Medicinal plant and its diversity on the basis of their habitat in Bangladesh:

There are 722 medicinal plants in our country (A. Gani1998). Most of them are grown in natural environment on the basis of their habitat they are classified into 5 types. Most of them are grown in different place and environment for their adaptation capacity.

In below a table is presented on the basis of their habitat.

Table-1.1: The list of medicinal plant grow in different habitat (Sadi, A., 2012)

SL No.	Plant Group	Habitat	Local Name of the plant
01	Plain land and crop land plant	Plain land cultivate land	Kanibashi, Ghatecochu, khadnli, Shialkata, Kukra, Ghagra, Dondokolosh, Kalomegh
02	Surrounding the path of home	Marginally high land there is no water	Bashok, Akondo, Tharkrri, Bonera, Talecochu, Chalta, Punornova,Dafura, Amloki, Tulsi, Kuch, Kashori
03	Forest an and Hill tracts plant	Deciduous forest, Hill track forest	Bormasri, Nota, Vui, Nallota, Nimaada
04	Fresh water and water logging land plant	Side of the canal, Hill Boure, Doba	Sadapata, Hizor, Halencha, Jolkolmi, Soale, Sapla, Saluk, Padma
05	Coastal areas plant	Coastal area and Mangrove forest	Hargoza, Panlots, Koromcha,Sadakolmi, junjuri, vola, Sadormala

1.8 Medicinal plant and its diversity on the basis of their utility in Bangladesh:

In below 30 medicinal plants are described with mention their local and scientific name, usable part and use:

Table-1.2: List of 30 medicinal plants in Bangladesh and their uses (Sadi, A., 2012)

SL No.	Local Name	Scientific Name	Family	Using Part	Control Disease Name
1	Apang	*Achyranthes Paniculata*	Amaranthaceae	Whole Plant	Dysentery, Constipation, Piles, Arthritis, Skin Disease.
2	Kalmegh	*Andrographis Pariculata*	Acanthaceae	Whole Plant	Metabolic Problem, Gastric, Fever, Worm Killer, Dysentery, Liver Disease, Strengthen.
3	Akondo	*Calotropis Procera*	Asclepiadaceae	Root, Leaf, Bark, Flower, Extract of Leaf	Ulcer, Tooth Pain, Chronic Dysentery, Cold, Asthma
4	Ontomul	*Hemidesmus Indicus*	Asclepiadaceae	Root & Whole Plant	Strength Increaser, Appetizer, Arthritis, Diabetes

5	Arjun	*Terminalia Arjuna*	Combretaceae	Bark	Heart Disease, Diarrhea, Piles, Tuberculosis
6	Ultokambol	*Abroma Augusta*	Sterculiaceae	Root, Bark & Leaf	Vaginal pail, Sexual Disease
7	Aloe vera	*Aloe Indica*	Liliaceae	Extract of leaf	Headache, Sexual Disease, Metabolic Problem, Fever
8	Centella/ Gotu Kola	*Centella Asiatica*	Apiaceae	Whole Plant	Metabolic Problem, Pain Killer, Diabetics, Ulcer, Chronic Dysentery, Anit-Coughing
9	Telkucha	*Eoccinia Cordifolia*	Cucarbitace	Leaf & Root	Diabetics, Cold, Appetizer, Ulcer
10	Kehraj/ False Daisy	*Eclipta Prostrata*	Asteraceae	Whole Plant	Headache, Cold, Control of Hair Losing
11	Swampweeds	*Hygrophilla Schulli*	Acanthaceac	Leaf, Seed, Steam	Control of Anger, Liver

					Disease, Ulcer, Bleeding, Removal of Stone from Kidney
12	Skunkvine/ Chinese Fever Vine	*Paedaria Foetida*	Rubiaceae	Leaf	DYSENTERY, Metabolic Disorder, Cold, Arthitis
13	Datura/ Devil's Trumpet	*Datura Metal*	Solanaceac	Root, Leaf, Seed	Pain Killer, Worn Killer, Poisonous
14	Bahera	*Terminalia Belerica*	Combretaceae	Fruit	Constipation, Diarrhea, Fever, Cough, Piles, Gastric, Heart Disease
15	Candle Bush	*Cassia Alata*	Fabaceae	Leaf	Skin Disease, Poisonous
16	Spearmint	*Mentha Viridis*	Lebiatae	Whole Plant	Metabolic Disorder, Gastric
17	Bashak/ Adulsa	*Adhatoda Vasica*	Acanthaceace	Leaf & Root	Cough, Asthma, Tuberculosis, Cold, Blood Refine

18	Sevenbark	*Hydrangea arborescons*	Saxifra Zaceac	Leaf, Flower, Fruit	Liver Disease, Adrenal Peptic Ulcer, Hormonal Disease
19	Brahmi/ Herb of Geace	*Becopa Monnieri*	Scrophulariaceae	Leaf	Heart Disease, Nurval Pressure, Asthma
20	Sankeroot/ Devil Pepper	*Rauvolfia Serpentina*	Apocynaceae	Leaf & Root	Blood Pressure,Brain Abnormal, Dysentery, Diarrhea, Pain Killer
21	Kurchi/ White Angel	*Holarrhena Antidysenterica*	Apocynaceae	Bark & Seed	Diarrhea, Dysentery, Worm Killer, Constipation, Intestinal Weakness
22	Neem	*Azadirachta Indica*	Meliaceae	Root, Leaf, Bark	Skin Disease, Worm Killer, Arthritis, Insecticide, Anti-vomiting, tooth Disease, Jaundice, Anti-

					viral
23	Flame Lily	*Gloriosa Superba*	Liliceae	Leaf & Steam	Arthritis, Adrenals Peptic, Ulcer
24	Shatamull	*Asparagus Racemosus*	Liliaceae	Leaf & Root	Fever, Dysentery, Diabetics
25	Bael	*Aegle Marmelos*	Rulaceace	Fruit	Dysentery, Diarrhea
26	Pineapple	*Ananas Comosus*	Bromeliaceae	Fruit & Leaf	Jaundice
27	Jambul	*Syzygium cumini*	Myrtaceae	Fruit & Seed	Diabetics, Dysentery
28	Climbing Hempvine	*Mikania Scandens*	Compositae	Leaf	Bleeding Control, Dysentery, Daud
29	Chinese Chaste tree	*Vitex Negundo*	Verbenaceae	Root &Leaf	Asthma, Arthritis, Fever
30	Durva Grass	*Cynodon Dactylon*	Grominae	Leaf	Bleeding Control, Skin Disease

Table-1.3: Percentage of Using Medicinal Plant in Several Types of Treatment (Sadi, A., 2012)

SL No	Types of Treatment	Percentage of Using Medicinal Plant
01	Folk	35
02	Aiurbadik	31
03	Unani	22
04	Homeopath	12

1.9 Name and Distribution of *Syzygium Samarangense*

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

Bengali Name : Jamrul

Common Name : Wax apple, royal apple, love apple.

Botanical Name : *Syzygium Samarangense*

Synonym : *Eugenia javanica*

Family : *Myrtaceae*

Figure 1.8: ***Syzygium Samarangense*** Fruits and Flowers

1.9.1 Botanic Description

Syzygium samarangense is a tree to 15 m tall, with short and crooked trunk, 25-50 cm diameter, often branched near the base and with wide, irregular canopy. Leaves opposite, elliptic to elliptic-oblong, 10-25 cm x 5-12 cm, coriaceous with thin margin, pellucid dotted, rather strongly aromatic when bruised; petiole thick, 3-5 mm long. Inflorescences terminal and in axils of fallen leaves, 3-30-flowered; flowers 3-4 cm in diameter, calyx-tube ca. 1.5 cm long, ventricose at apex, lobes 3- 5 mm long; petals 4, orbicular to spathulate, 10-15 mm long, yellow-white; stamens numerous, up to 3 cm long; style up to 3 cm long. Fruit a berry, broadly pyriform, crowned by the fleshy calyx with incurved lobes, 3.5-5.5 cm x 4.5-5.5 cm, light red to white; flesh white spongy, juicy, aromatic, sweet-sour in taste. Seeds are 0-2, mostly suppressed, globose, up to 8 mm in diameter.

1.9.2 Biology

Shoot growth proceeds in flushes which are more or less synchronous, depending on the climate. The juvenile period lasts for 3-7 years. Bearing of clonal trees starts after 3-5 years. There are definite flowering seasons, often two, sometimes three in a year, but the timing varies from year to year. Wax jambu commonly flowers early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 30-40 days after anthesis. [Orwa et al, (2009)]

1.9.3 Ecology

The trees are at home in fairly moist tropical lowlands up to 1200 m elevation. Wax jambu grows best in areas with a fairly long dry season. This does not mean that this species is drought-resistant. The species require a reliable water supply and are often planted along streams or ponds. (Orwa et al, 2009)

1.9.4 Biophysical Limits Altitude

Soil types: The trees prefer heavy soils and easy access to water instead of having to search for water in light deep soils.

1.9.5 Documented Species Distribution

Native: Fiji, India, Indonesia, Malaysia

Exotic:

Figure1.9: Geography of ***Syzygium Samarangense***

The map above shows countries where the species has been planted. It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site. (Orwa et al, 2009)

1.9.6 Products Food

The tree is grown for their fruit, which substitute for one another in the marketplace. It is not easy to distinguish between the various *S. aqueum* and *S. samarangense* fruits. The ripe fruit is

sweet and is mainly eaten fresh. In Indonesia wax jambu is used in fruit salads ('rujak') and they are also preserved by pickling ('asinan'). Eighty per cent or more of the fruit is edible. The composition the species per 100 g edible portion: water more than 90%, protein 0.3 g, fat none, carbohydrates 3.9 g, fibre 1 g, vitamin A 253 IU, vitamin B1 and B2 traces, vitamin C 0.1 mg, energy value 80 kJ/100 g (analysis for wax jambu in Thailand). [Orwa et al,(2009)]

1.9.7 Medicine

Various parts of the tree are used in traditional medicine, and some have in fact been shown to possess antibiotic activity. Timber: The wood is reddish, hard and grows to dimensions large enough for construction purposes.

1.9.8 Tree Management

Tree spacing ranges from 8-10 m. The trees receive little attention after the first year or two when manuring, weeding, mulching and watering ensure rapid increase of tree volume. Trees which bear well benefit from compound fertilizers applied after harvest and supplemented with a top dressing as soon as the inflorescences are being formed. There appears to be no experience with pruning or fruit thinning. The fruits have a thin skin and are delicate; they need to be picked by hand twice a week and handled with care. The fruit should be consumed or preserved within a few days from harvest. A five-year-old wax jambu may yield 700 fruit. [Orwa et al,(2009)]

1.9.9 Pests and Diseases

There are no specific recommendations for crop protection, but the incidence of pests and diseases certainly warrants a study of the causal organisms and their control. (Orwa et al, 2009)

CHAPTER

TWO

LITERATURE REVIEW

2.1 Anti-diarrheal Activity of *S. samarangenese*, Java apple

The hexane extract of *S. samarangense* was found to dosedependently (10-3000 microg/mL) relax spontaneously contracting isolated rabbit jejunum. When tested for a possible calcium channel blocking (CCB) activity, the extract (10-1000 microg/mL) relaxed the high K+-induced contractions and also decreased the Ca++ dose-response curves in a dosedependent manner (30-100 microg/mL), confirming the CCB activity. The flavonoids isolated from the hexane extract were tested for a possible spasmolytic activity. All flavonoids, showed dosedependent (10-1000 microg/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 diarrhoea. (peter, T., et al, 2011)

2.2 Anti-bacterial activity of *S. samarangenese*, Java apple

The methanolic and the petroleum ether extracts of S. samarangense exhibited significant antimicrobial activity on certain pathogens. The minimum inhibition and minimum bacterial/fungal concentrations were determined by microdilution 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. (peter, T., et al, 2011)

2.3 Cytotoxic chalcones and antioxidants from the fruits of *Syzygium samarangense* (Wax Jambu)

Bioassay-guided fractionation of the methanolic extracts of the pulp and seeds of the fruits of *S. samarangense* (Blume) Merr. And L.M. Perry led to the identification of four cytotoxic compounds and eight antioxidants on the basis of HPLC-PDA analysis, MS and various NMR spectroscopic techniques. Three C-methyl 6′-methoxychalcone(1),2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone(1), 2′,4′-dihydroxy-3′-methyl-6′-methoxychalcone (stercurensin 2), and 2′,4′- dihydroxy-6′-methoxychalcone (cardamonin 3), were isolated and displayed cytotoxic activity (IC50=10,35 and 35 μM, respectively) against the SW-480 human colon cancer cell line. Also a number of known antioxidants were obtained including six quercetin glycosides: reynoutrin, hyperin, myricitrin, quercitrin, quercetin and guaijaverin, one flavanone: (S)-pinocembrin, and two phenolic acids: gallic acid and ellagic acid. [Simirgiotis, et al, (2008)]

2.4 Triterpenoids and chalcone from *Syzygium samarangense*

A new triterpine, methyl 3-epi-betulinate in its native form and 4′,6′- dihydroxy-2′-methoxy-3′,5′-dimethyl chalcone along with ursolic acid, jacoumaric acid and arjunolic acid have been isolated from the aerial parts of Syzygium samarangense. (Srivastava, R., et al, 1995)

2.5 Antihyperglycemic Activities of Leaves of Three Edible Fruit Plants (*Averrhoa carambola, Ficus hispida* and *Syzygium samarangense*) of Bangladesh

*Averrhoa carambola L. (*Oxalidaceae), *Ficus hispida L.F. (*Moraceae), and *Syzygium samarangense* (Blum) Merr. And L.M. Perry (Myrtaceae) are three common plants in Bangladesh, the fruits of which are edible. The leaves and fruits of *A. carambola* and *F. hispida* are used by folk medicinal practitioners for treatment of diabetes, while the leaves of *S. samarangense* are used treatment of cold, itches and waist pain. Since scientific studies are absent on the anti-hyperglycemic effects of the leaves of the three plants, it was the objective of the present study to evaluate the anti-hyperglycemic potential of methanolic extract of leaves of the plants in oral glucose tolerance tests carried out with glucose-loading mice. The extracts at different doses were administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (P.O.) using glucose oxidize method. Significant oral hypoglycemic activity was found with the extracts of leaves of all three plants tested. The fall in serum glucose levels were dose dependent for every individual plant, being highest at the highest dose tested of 400 mg extract per kg body weight. At this dose, the extracts of *A. carambola, F. hispida* and *S. samarangense* caused, respectively 34.1, 22.7 and 59.3% reductions in serum glucose levels when compared to control animals. The standerd anti-hyperglycemic drug, glibenclamide, caused a 57.3% reduction in serum glucose levels of *s. samarangense* proved to be the most potent in demonstrating anti-hyperglycemic effects. The result validates the folk medicinal uses of *A. carambola, F. hispida* in the treatment of diadetes and indicates that the leaves of *S.samarangense* can also possibly be used for amelioration of diabetes induced hyperglycemia. (Shahreen, S., et al, 2016)

2.6 Antioxidant activities of *S.* samarangenese, Java apple

Antioxidant activitiy 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 IC50 of the ethanolic extract S. samarangense was 200 microg/mL. This indicates the fruit is beneficial to human health. (peter, et al, 2011)

2.7 Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits

Many species of Myrtaceae are cultivated in home gardens throughout the tropics for their edible fruit, and have been used in traditional medicine to treat several inflammatory conditions. Fruit phenolics are important dietary antioxidant and anti-inflammatory constituents. We have investigated the antiradical activity, total phenolic content (TPC), and total anthocyanin content (TAC) of 14 underutilized Myrtaceae fruits, namely *Eugeniaaggregata, E.brasiliensis, E. luschnathiana, E. reinwardtiana, Myrciariacauliflora, M. dubia, M. vexator, Syzygiumcumini, S. curranii, S. jambos, S. javanicum, S. malaccense, S. samarangense,and S. samarangense var. Taiwanpink*. An HPLC-PDA method was developed to quantify the amounts of cyanidin 3-glucoside (1), delphinidin 3-glucoside (2), ellagic acid (3), kaempferol (4), myricetin (5), quercetin (6), quercitrin (7), and rutin (8) present in MeOH extracts of the fruit. TPC ranged from 3.57 to 101 mg/g, TAC ranged from undetectable to 12.1 mg/g, and antiradical activity, measured as DPPH˙ IC50, ranged from very active (19.4 μg/ml) to inactive (389 μg/ml). (Reynertson, et al, 2008)

2.8 Antihyperglycaemic flavonoids from *Syzygium samarangense* (Blume) Merr. and Perry

2,4-Dihydroxy-3,5-dimethyl-6-methoxychalcone1, it's isomeric flavanone 5-O-methyl-4-desmethoxymatteucinol 2 and 2,4-dihydroxy-6-methoxy-3-methylchalcone 3 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 [sol] g mouse, 1 and 2 significantly (α=0.05) lowered the blood glucose levels (BGLs) in glucose-hyperglyceaemic mice when administered 15 min after a glucose load. When co-administered with glucose, only 1 showed a significant lowering of BGLs showerd a significant lowering of BGLs 45 min after its oral administration. Whem administered 15 min before glucose, none of the flavonoids showed a positive effect. Only 1 decreased significantly, at α=0.05, the BGLs of alloxan-daibetic mice at t=90-150 min. (Resurreccion-Magno, et al, 2015)

2.9 New and rare flavonol glycosides from leaves of *Syzygium samarangense*

Tow flavonol glycoside have been isolated and characterized from leaves of s. samarangense. One is the rare mearnsitrin while the second 2-C-methyl-5-O-galloylmyricetin-3-O-a-L-rhamnopyranoside is new. (Nair, A., et al, 1999)

2.10 Anticholinesterase Activity of *S. samarangenese*, Java apple

The actual inhibitory assay involves the additionof 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 DTNB stock 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. (peter, T.et al, 2011)

2.11 Immunopharmacological Activity of *S. samarangenese*, Java apple

The flavonoids isolated from S. samarangense were evaluated for immunopharmacological activity. Human peripheral blood mononuclear cells (PBMC) were used as target cells, and cell proliferation was determined by 3H-thymidine uptake. Among the flavanoids, (-)-strobopinin (1), myricetin 3-O-(2''-O-galloyl)-alpha-rhamnopyranoside (2), (-)- epigallocatechin 3- O-gallate (3) and myricetin 3-O-alpha-rhamnopyranoside (4) showed inhibitory potency on PBMC proliferation activated by phytohemagglutinin (PHA). The IC50 values of compounds 1, 2, 3, and 4 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 IFNgamma production in PBMC in a dose-dependent manner. (peter, T. et al, 2011)

2.12 Evaluation of Analgesic, Anti-Inflammatory and CNS Activities of the Methanolic Extract of Syzygium samarangense Leave

Syzygium Samarangense is a potential medicinal drug. We aimed to evaluate the analgesic, antiinflammatory 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 *Syzygium Samarangense* 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)

2.13 Physiochemical and Phytochemical Properties of Wax Apple (*Syzygium samarangense* [Blume] Merrill & L. M. Perry var. Jambu Madu) as Affected by Growth Regulator Application

This study represents the first paper of the effects of growth regulators on the physiochemical and phytochemical properties of the wax appel fruit, a widely cultivated fruit tree in southeast Asia. Net photosynthesis, sucrose phosphate synthesis (SPS) activity,peel color, fruit firmness, juice content, pH value, total soluble solids (TSSs) and suger acid ratio were all significantly increased in growth regulators (PGRs) treated fruits. The application of gibberellins (G A_3), naphthalene acetic acid (NAA) and 2.4-dichlorophenoxy acetic acid significantly reduced titratable acidity and increased total sugar and carbohydrate content compared to the control. Yhe 50 mg/L GA_3, 10 mg/L NAA and % mg/L 2,4-D treatments produced the greatest increases in

phenol and flavonoid content; vitamin C content was also higher for these treatments. PGR treatment significantly affected chlorophyll, anthocyanin and carotene content and produce higher phenylalanine ammonia lyase (PAL) and antioxidant activity levels. There was a positive correlation between peel color, TSS and antioxidant activity and both phenol and flavonoid content and PAL activity and anthocyanin formation. A taste panel assessment was also performed, and the highest scores were given to fruits that had been treated with GA_3. The study showed that application of 50 mg/L GA_3, 10 mg/L NAA and 5 mg/L 2,4-D once a week from bud development to fruit maturation increased the physiochemical and phytochemical properties of wax apple fruits. (Khandakar, et al, 2012)

2.14 Fraction from*Syzygium samarangense* Fruit Extract Ameliorates Insulin Resistance via Modulating Insulin Signaling and Inflammation Pathway in Tumor Necrosis Factor α-Treated FL83B Mouse Hepatocytes

Inflammation is associated with the development of insulin resistance in Type 2 diabetes mellitus. In the present study, mouse FL83B cells were treated with tumor necrosis factor-alpha (TNF-α) to induce insulin resistance, and then co-incubated with a fraction from wax apple fruit extract (FWFE). This fraction significantly increased the uptake of the nonradioactive fluorescent indicator 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG) in insulin resistant cells. Western blot analysis revealed that, compared with the TNF-α-treated control group, FWFE increased the expression of the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), protein kinase B (Akt/PKB), phosphatidylinositol-3 kinase (PI3K), and glucose transporter 2 (GLUT-2), and increased IR tyrosyl phosporylation, in insulin resistant FL83B cells. However, FWFE decreased phosphorylation of c-Jun *N*-terminal kinases (JNK), but not the expression of the intercellular signal-regulated kinases (ERK), in the same cells. These results suggest that FWFE might alleviate insulin resistance in TNF-α-treated FL83B cells by activating PI3K-Akt/PKB signaling and inhibiting inflammatory response via suppression of JNK, rather than ERK, activation. (Shen, S., et al, (2012)

2.15 Application of Girdling for Improved Fruit Retention, Yield and Fruit Quality in *Syzygium samarangense* under Field Conditions

The study was undertaken to investigate the effects of different girdling techniques on the yield and quality of wax jambu fruits (Syzygium samarangense). Physiological and biochemical parameters were monitored at one week intervals during the three successive growth period from January, 2009 to May, 2010, using I-25%, C, V shaped, I-50% and 100% girdling. Girdling was applied three weeks before flowering every season. It was observed that the C-shaped girdling technique significantly enhanced the inflorescence development and produced the best results with regard to the fruit retention, fruit size, leaf chlorophyll and drymatter in comparison to the control and the other girdling techniques employed. Furthermore, C-shaped girdling enhanced faster fruit growth producing the best final fruit length and diameter, in addition to significantly increased number of fruits and mean fruit weight. It was also observed that I-50% girdling increased the L\D ration of fruit. I-shaped girdling increased the photosynthetic yield and dry matter content in the fruits compared to the control. With regard to fruit quality, the application of C-shape girdling increased total sugars, total phenolics and anthocyanins content in the fruits by 87, 28 and 138%, respectively compared to the control treatment. V-shape girdling increased the total flavonoids 150% more than control fruits. Girdling practices increased the antioxidant activity in the fruits. From this study, it can be concluded that girdling applied before flowering enhanced inflorescence development, increased yield and quality of wax jambu fruits under field conditions. (Nasrulhaq Boyce, A. 2011)

2.16 Hypotriglyceridemic and hypoglycemic effects of vescalagin from Pink wax apple [*Syzygium samarangense* (Blume)] in high-fructose diet-induced diabetic rats

Vescalagin, an active component from Pink wax apple [*Syzygium samarangense*(Blume) Merrill and Perry cv. Pink] fruit, with glucose uptake enhancing ability in insulin-resistant FL83B mouse hepatocytes, as shown in our previous study, was further evaluated for its 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 present study reveals that vescalagin could have therapeutic value against diabetic progression via its anti-hypertriglyceridemic and anti-hyperglycemic effects. (Shen, S. et al, 2013)

CHAPTER THREE

METHODS AND MATERIALS

3.1 Collection & Preparation of Plant Material

Plant sample (Leaves) of *Syzygium Samarangense* was collected from Dohar, Dhaka in March 2016. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves 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 (5 liters) 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 filter paper 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.

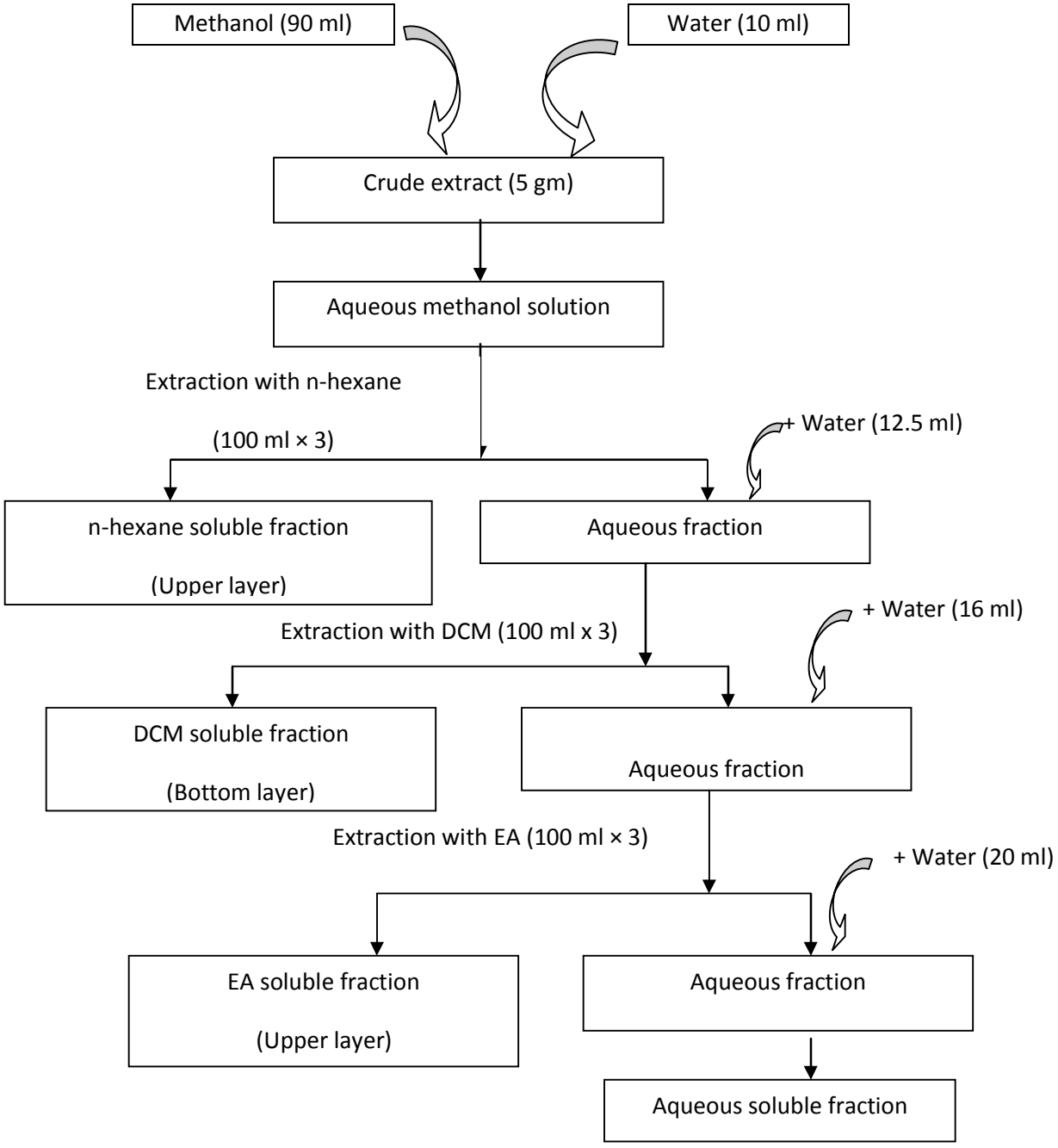


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Syzygium Samarangense* leaves

3.4.1 Partition with n-Hexane

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 n-hexane fraction was then air dried for solid residue.

3.4.2 Partition with Dichloromethane

To the mother solution left after partitioning with n-hexane, 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 was then air dried for solid residue.

3.4.3 Partition with Ethyl acetate

To the mother solution that left after washing with n-hexane, 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 n-hexane 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.

3.4.5 Collection of Ethyl Acetate Fraction

After partitioning the mother solution with the four different solvents the ethyl acetate fraction was collected and air dried. This ethyl acetate was further investigated for different pharmacological properties (antioxidant, cytotoxic and antimicrobial).

3.5 Antioxidant Activity

3.5.1 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 antioxidants 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 antioxidative effects have rarely been carried out. The purpose of this study was to evaluate extractives of Opuntia elatior as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity. 50 Cytotoxic and Antioxidant activity in aqueous fraction of Opuntia elatior extract

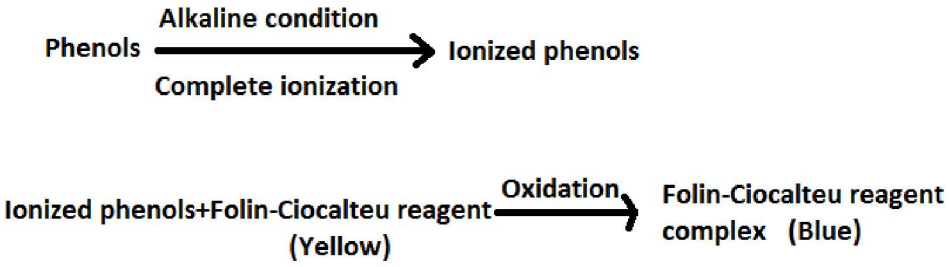
3.5.1.1 Principle

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 3.1: Composition of 100 mg Folin-Ciocalteu Reagent

Water	57.5 ml
Lithium Sulfate	15.0 mg
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

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 heteropolyphosphotunstates - molybdates. Sequences of reversible oneor two-electron reduction reactions lead to blue species, possibly (PMoW11O40) 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).



3.5.1.2 Apparatus & Reagents:

Table 3.2: Apparatus and reagents used for total phenolic content

Folin-Ciocalteu reagent (10 fold diluted)	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Na2CO3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200 µl)
Distilled water	Cuvette

3.5.1.3 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 Na2CO3 (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 Opuntia elatior aqueous 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.

3.5.2 Total Flavonoid Content

3.5.2.1 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 C et al., 2002). Flavonoid (Extract) + $AlCl_3$ (reagent) = Formation of flavonoid-aluminium complex (λ_{max} = 510 nm)

3.5.2.2 Apparatus & Reagents

Table 3.3: Apparatus and reagents used for total flavonoid content

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

3.5.2.3 Procedure

Preparation of 10% Aluminium Chloride (Alcl3) Solution: 10 mg of AlCl3 was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of 4% NaOH Solution: 4 mg 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: 5 mg of NaNO2 was taken into a 100 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. The experimental concentrations were prepared from this stock solution.

Table 3.4: Preparation of standard solution

Concentration (µg/ml)	**Solution taken from stock solution (ml)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
0	0.0	5	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 ml 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 NaNO2was added and incubated for 6 minutes. 10% AlCl3 was added and incubated for 6 minutes. 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. Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank.

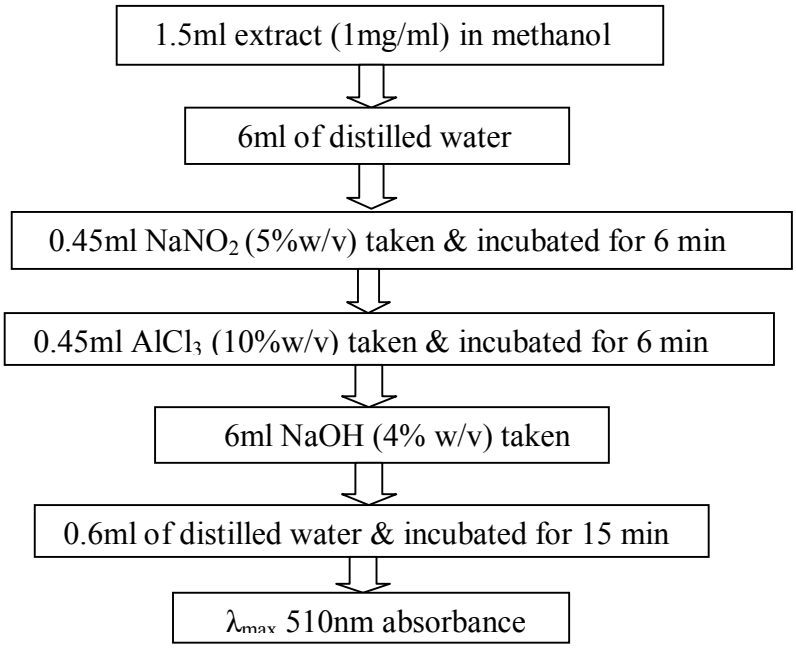


Figure 3.3: Schematic diagram of preparation of extract solution

Preparation of blank solution

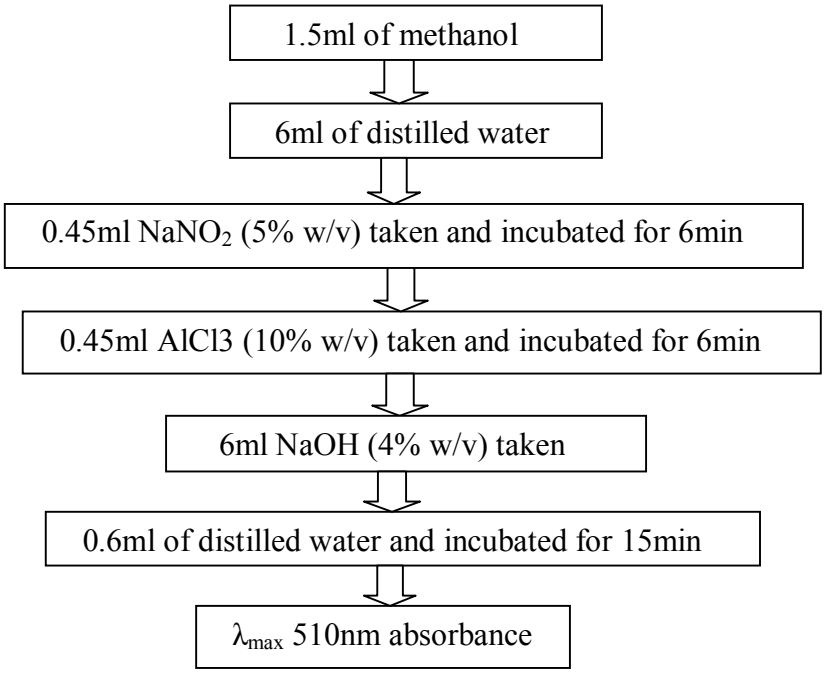


Figure 3.4: Schematic diagram of preparation of blank solution

3.6 Brine Shrimp Lethality Bioassay

3.6.1 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- Artemiasalina) 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 for 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 and Nuneza, 2013; Rishikesh et al., 2013).

3.6.2 Apparatus & Reagents

Table 3.5: Apparatus and reagents for Brine shrimp lethality bioassay

Artemiasalina 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

3.6.3 Procedure

3.6.3.1 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 38gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000ml by distilled water in a 1000ml beaker for Artemiasalina hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the pH 8.4 as sea water.

3.6.3.2 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 Artemiasalina 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 18-24hr. 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 5ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay.

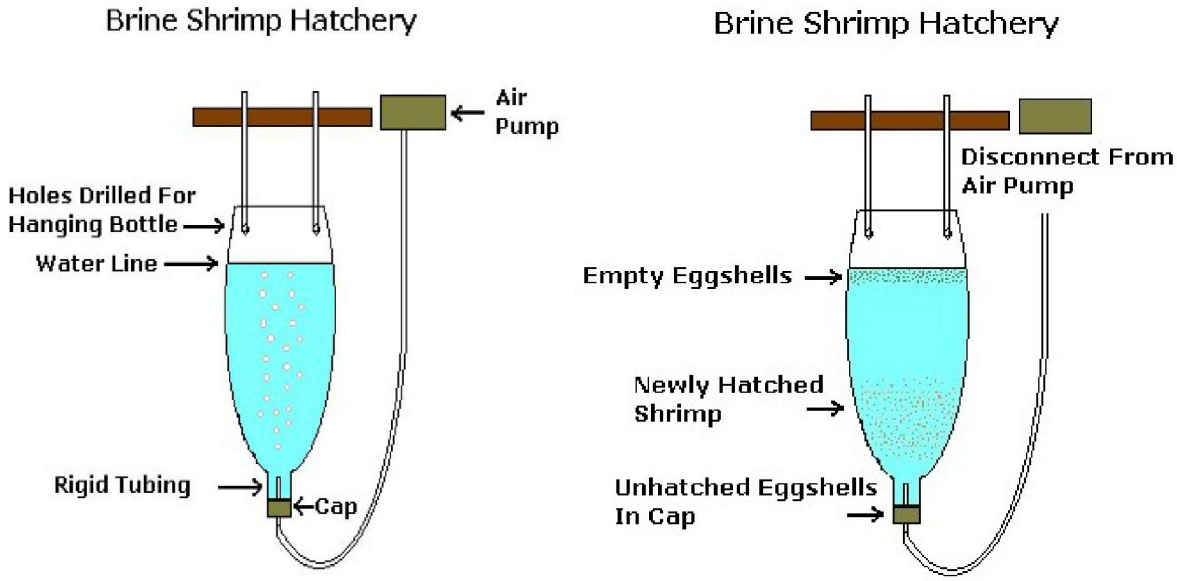


Figure 3.5: Brine shrimp Hatchery

3.6.3.3 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.

3.6.3.4 Preparation of The Test Samples of Experimental Plant

All the test samples of 4mg were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions. Then 100µl of solution was taken in test tube each containing 5ml 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 100µl sample was added to test tube and fresh 100µl DMSO was added to vial. Thus the concentrations of the obtained solution in each test tube were 400µg/ml, 200µg/ml, 100µg/ml,

50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml for 10 dilutions.

3.6.3.5 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 20µg/ml. From that stock solution serial dilutions are made using DMSO to get 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml. Then ten living brine shrimp nauplii in 5ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.6.3.6 Preparation of the Negative Control Group

100µl of DMSO was added to the pre-marked test tube containing 5ml 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.

3.6.3.7 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.

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*

Ethyl acetate 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 organisms were 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 bacteria	*Bacillus sereus* *Bacillus subtilis* *Bacillus megaterium* *Staphylococcus aureus*
Gram negative bacteria	*Escherichia coli* *Salmonella typhi* *Salmonella paratyphi* *Vibrio parahaemolyticus* *Vibrio mimicus* *Shigelladysenteriae*
Fungi	*Candida albicans* *Aspergillusniger*

3.7.5 Procedure

3.7.5.1 Preparation of the Medium

To prepare required volume of this medium, 5.6gm 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 3.6: 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 1210C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

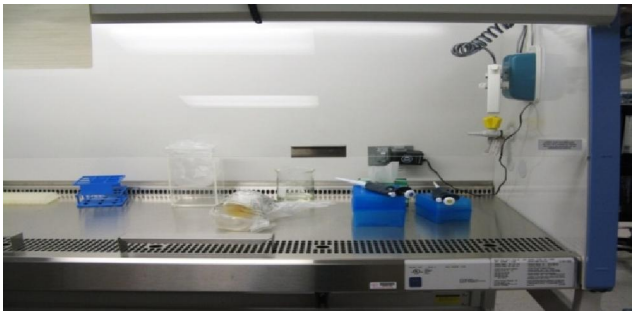


Figure 3.7: Laminar hood

3.7.5.3 Preparation of the Test Plate

The test organisms were transferred from the subculture to petridish containing about 10 ml of melted and sterilized agar medium. The bacterial and fungal suspension was taken by a loop mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial suspension. Then the bacterial sample is applied to the petri dish with the help of this cotton bud.

3.7.5.4 Preparation of Discs

Three types of discs were used for antimicrobial screening.

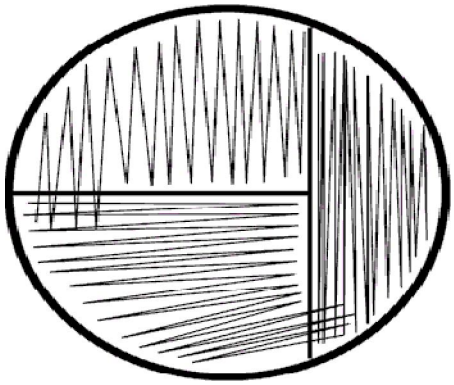


Figure 3.8: Preparation of filter paper discs

- **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, azithromycin (30µg/disc) disc was used as the reference.
- **Blank Discs:** These were used as negative controls which ensure that the residualϖ 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 knownϖ 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 Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

3.7.5.6 Application of Test Samples

Standard azithromycin 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 3.9: Incubator

3.7.5.8 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.

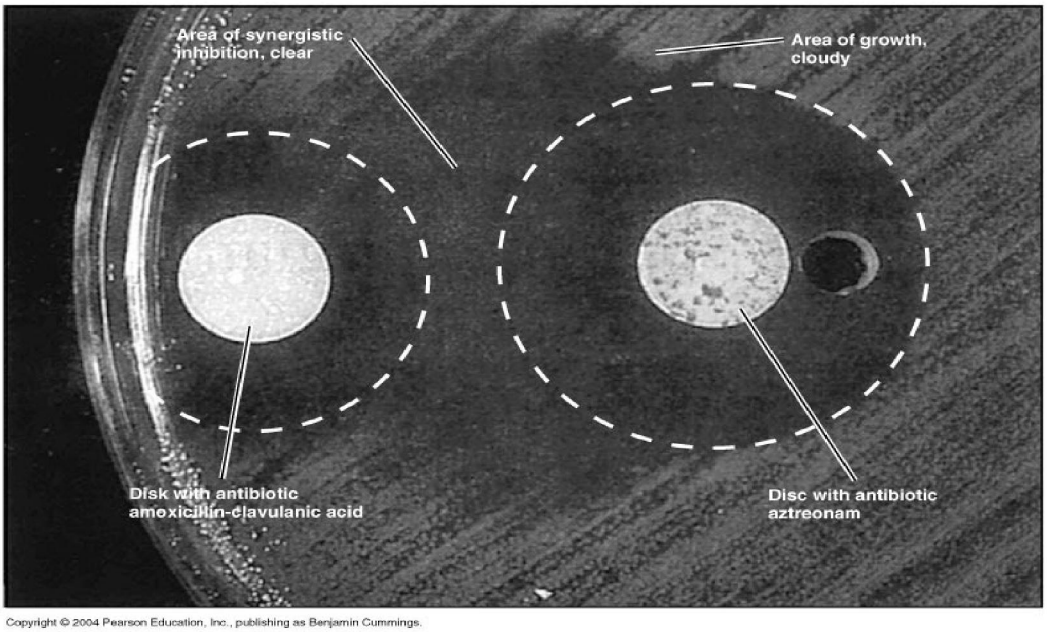


Figure 3.10: Clear zone of inhibition

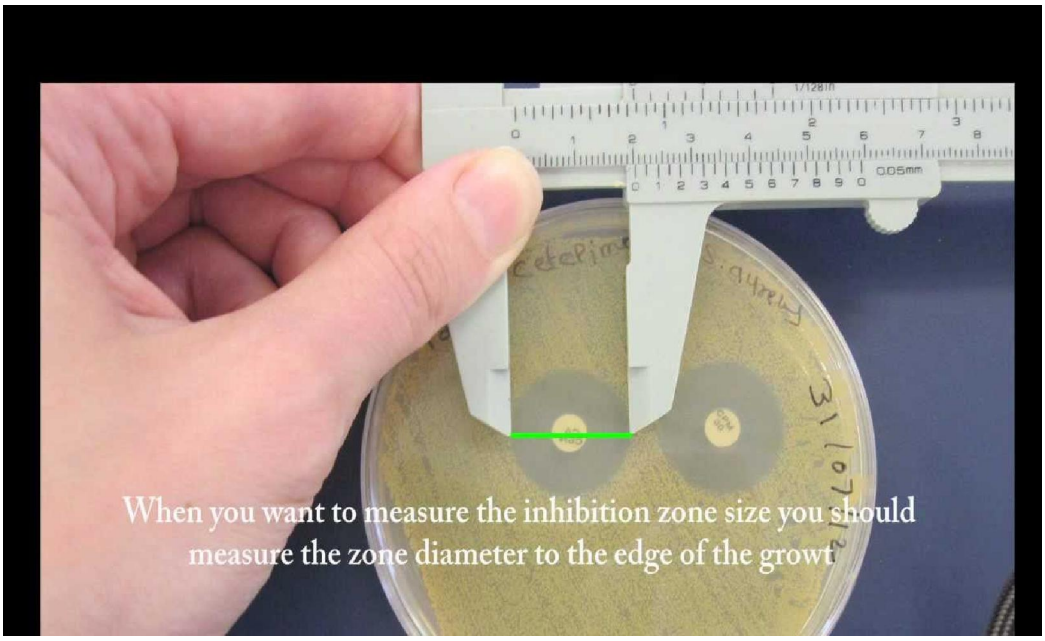


Figure 3.11: Determination of clear zone of inhibition

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Result of Antioxidant Tests

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 ethyl acetate fraction of *Syzygium Samarangense* leaves extract was determined by following methods-

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

4.1.1 Result of total Phenolic Content

The ethyl acetate extract of leaves of *S. samarangense*were subjected to determine total phenolic content. Ascorbic acid was used as reference standard.

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, as shown in Figure 4.1. This linear curve was considered as a standard curve.

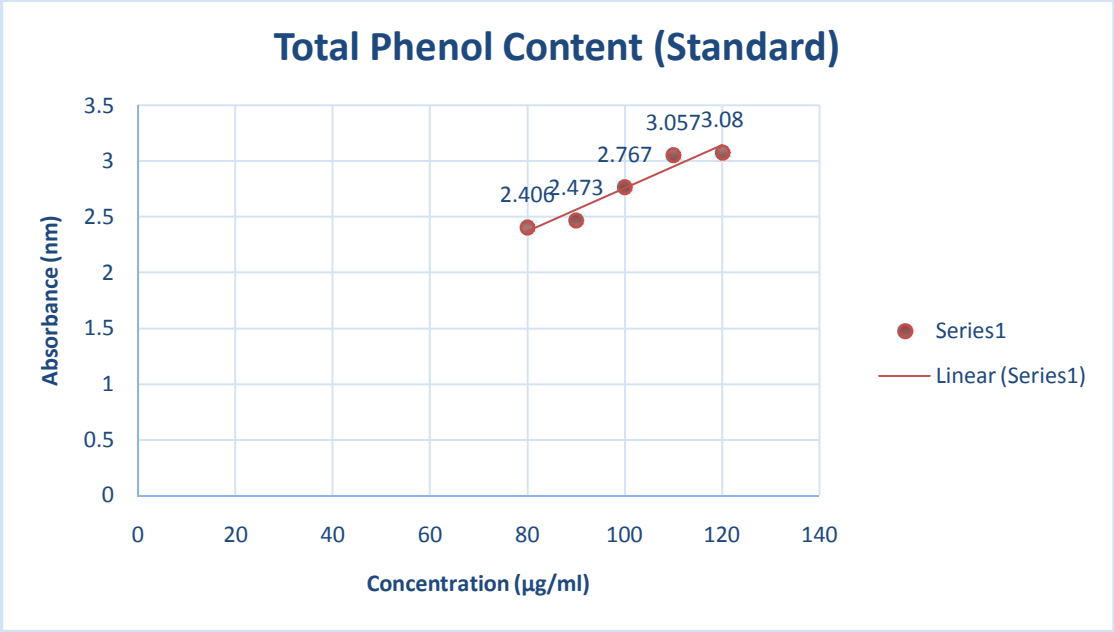


Figure 4.1: Graphical Representation of assay of phenolic content of Ascorbic Acid.

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

Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared 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 ethyl acetate 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)**
Ethyl acetate fraction of *S. samarangense*	2	3.922	160.48

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 ethyl acetate 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, 160.48 mg of AAE/gm of dried extract of phenol content was found in the ethyl acetate fraction of *S. samarangense.*

4.1.2 Result of Total Flavonoid Content

The ethyl acetate 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 quertecin 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

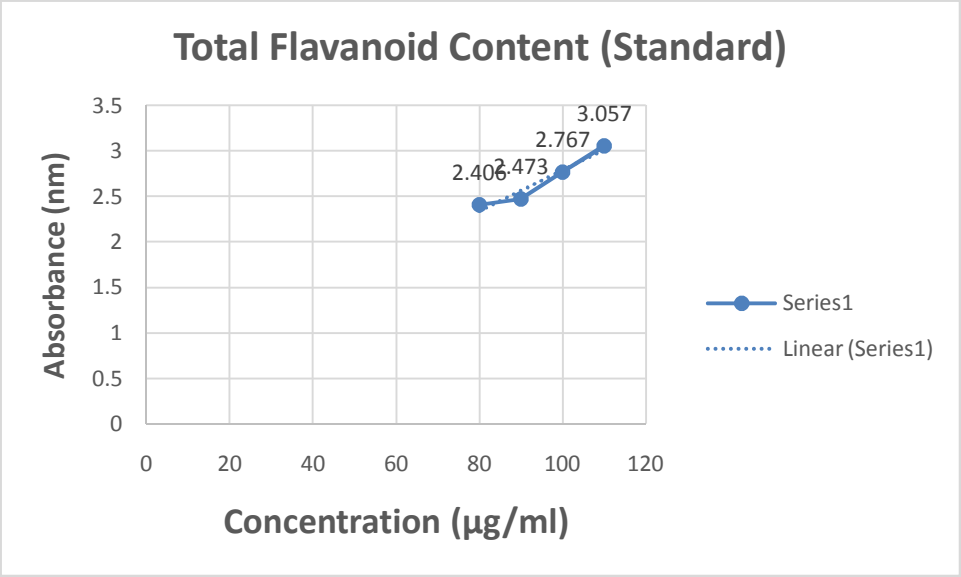


Figure 4.2: Graphical representation of Flavonoid content of quercetin.

4.1.2.2. Total Flavonoid Content Present in ethyl acetate 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 ethyl acetate 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)
ethyl acetate fraction of *S. samarangense*	5	0.056	1.302

4.1.2.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in flavonoid content. Absorbance of the

ethyl acetate 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, 1.302 mg of Quercetin/gm of dried extract of flavonoid content was found in the ethyl acetate fraction of *S. samarangense* (leaves).

4.2 Result of Antimicrobial Test

The antimicrobial activities of ethyl acetate fraction of *S. samarangense* leaves extract were subjected in the study against various Gram positive bacteria, Gram negative bacteria and fungi. The ethyl acetate 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 ethyl acetate Fraction

Table 4.5: Antimicrobial activity of standard sample (Ciprofloxacin) and ethyl acetate fraction

Type of microorganism		**Zone of inhibition (mm)**	
		Standard sample	**ethyl acetate fraction**
Gram positive bacteria	*Bacillus sereus*	31	6
	Bacillus subtilis	31	7
	Bacillus megaterium	30	0
	Staphylococcus aureus	31	7
Gram negative bacteria	*Escherichia coli*	30	8
	Salmonella typhi	30	9
	Salmonella paratyphi	32	9
	Vibrio parahaemolyticus	30	9
	Vibrio mimicus	30	7
	Shigella dysenteriae	33	7
	Candida albicans	30	7

Fungi	*Aspergillus niger*	30	6

4.2.2 Discussion

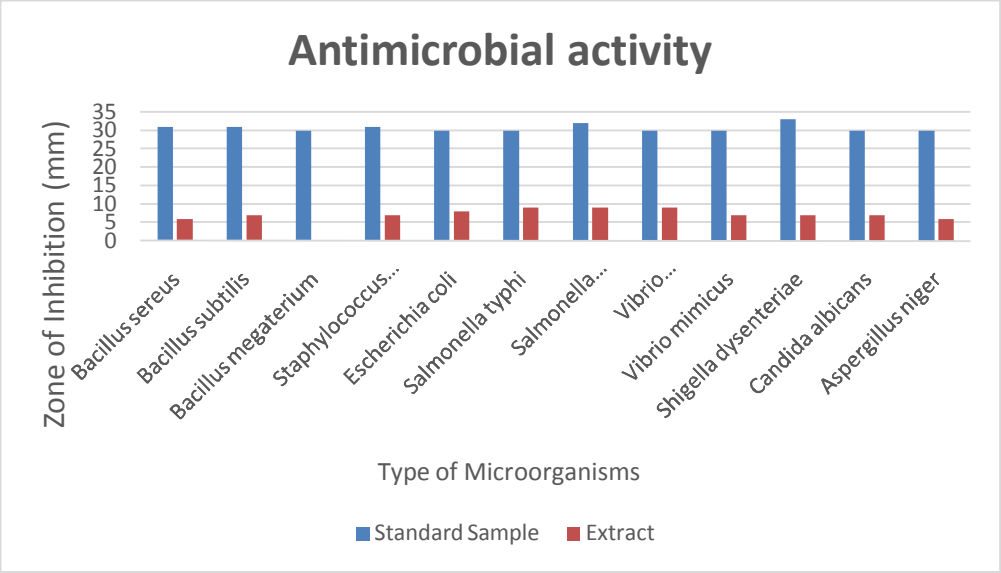


Figure 4.3: Comparison of antimicrobial activity between standard and extract

Ethyl acetate *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 ethyl acetate 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 *Salmonella typhi, Salmonella paratyphi, Vibrio parahaemolyticus* (9 mm) comparable to the standard (30, 32 and 30 mm respectively).

4.3 Result of Brine Shrimp Lethality Bio-Assay

The ethyl acetate 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 ethyl acetate 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	

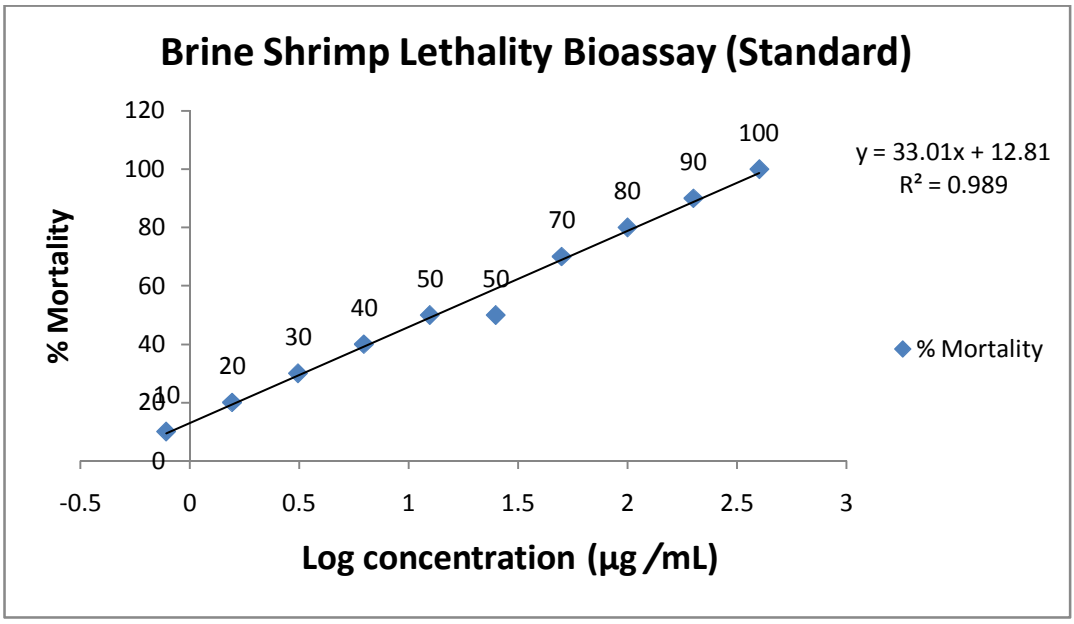


Figure 4.4: % Mortality and Predicted Regression Line of Tamoxifen (standard)

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

Table 4.7: Results of the bioassay in ethyl acetate 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	1	9	90	
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	2	8	80	
5	25	1.398	4	6	60	3.45
6	12.5	1.097	4	6	60	
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	8	2	20	

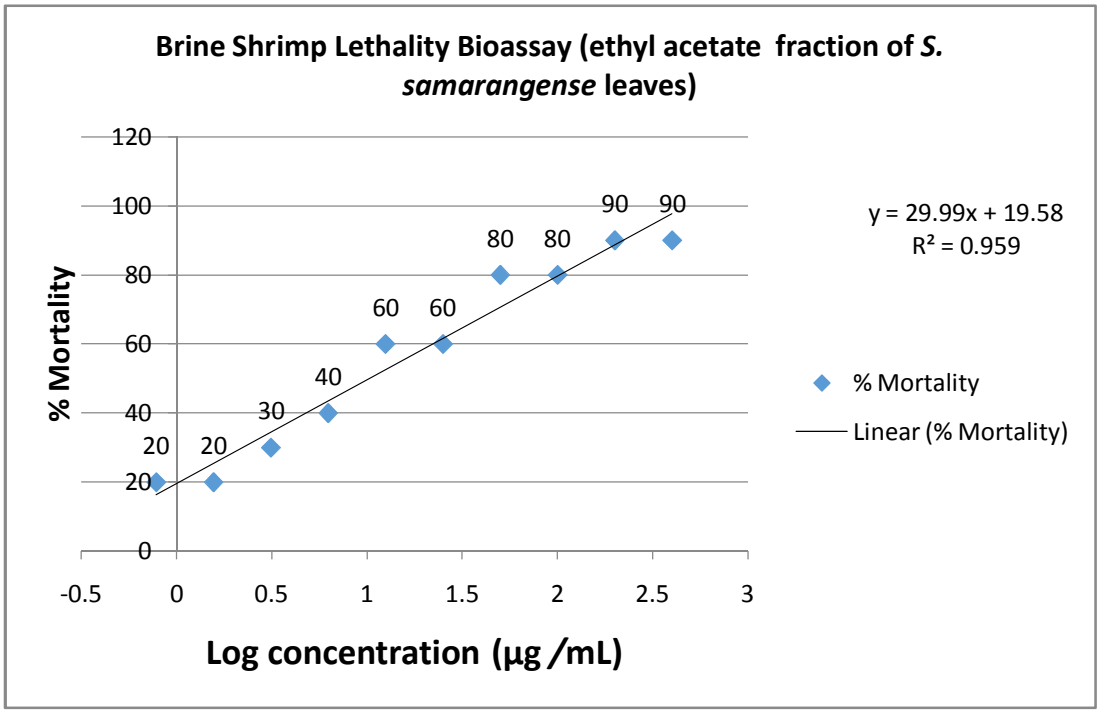


Figure 4.5: % Mortality and Predicted Regression Line in DCM fraction of *S. samarangense* (leaves)

4.3.3. Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional 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 in Table 4.7.

Table 4.8: Cytotoxic activity of Tamoxifen and ethyl acetate fraction of *S. samarangense* (leaves)

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml)
Standard (Tamoxifen)	y = 33.018x + 12.813	0.9891	13.38
Ethyl acetate fraction	y = 29.998x + 19.58	0.9597	3.45

In this investigation, standard and ethyl acetate fraction exhibited cytotoxic activities with the LC_{50} values at 13.38µg/ml and 3.45µg/ml respectively as shown in Table 4.8. LC_{50} value of *S. samarangense* (leaves) in ethyl acetate fraction showed more activity of it than Tamoxifen. Further investigation is needed to confirm the activity.

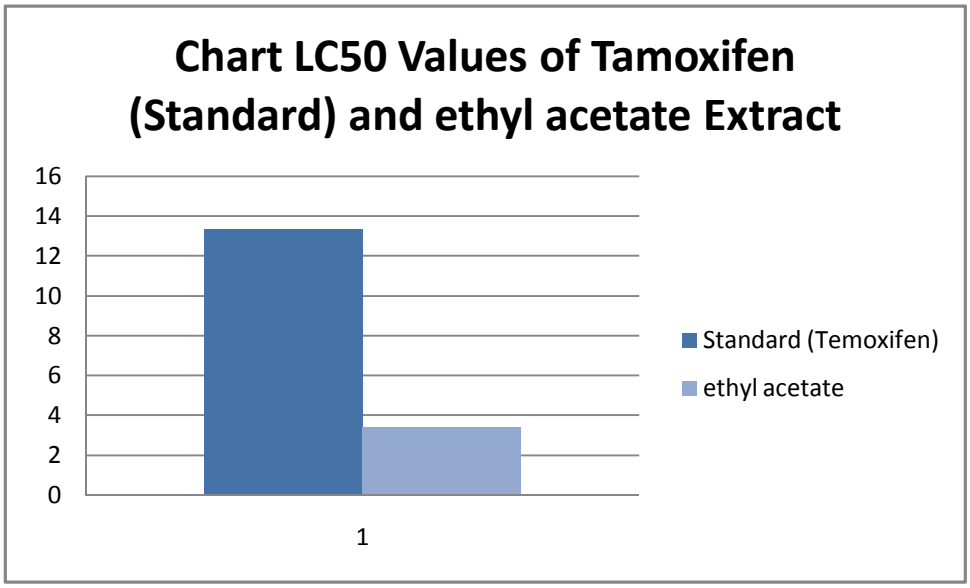


Figure 4.6: Comparison between LC50 values of standard and ethyl acetate extract

CHAPTER FIVE

CONCLUSION

5.1 Conclusion

As the literature review suggests, the presence of several phytochemical compounds in *Syzygium Samarangense* makes the plant pharmacologically active. The present study showed that it has poor antioxidant activity.

The ethyl acetate extract possesses cytotoxic activity that could be a better treatment in tumor as well as cancer.

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.

The medicinal values of the leaves of this plant may be related to their phytochemical constituent. So, further investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be tested. It will help in the development of new novel and safe drugs for the treatment of various diseases.

CHAPTER SIX

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