Cytotoxic, Antimicrobial and Antioxidant activity of aqueous fraction of *Ficus racemosa* 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

Nipa Rani Devi

ID: 2011-3-70-039

Department Of Pharmacy

East West University

DECLARATION BY THE CANDIDATE

I, Nipa Rani Devi, hereby declare that this dissertation, entitled **"Cytotoxic, Antimicrobial and Antioxidant activity of aqueous fraction of *Ficus racemosa* 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.

Nipa Rani Devi

ID: 2011-3-70-039

Department of Pharmacy

East West University

Aftabnagar, Dhaka

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"Cytotoxic, Antimicrobial and Antioxidant activity of aqueous fraction of *Ficus racemosa* leaves extract"** is a bonafide research work done, under my guidance and supervision by Nipa Rani Devi (ID: 2011-3-70-039), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

_____

Abdullah-Al-Faysal
Lecturer
Department of Pharmacy,
East West University, Dhaka

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the thesis entitled **"Cytotoxic, Antimicrobial and Antioxidant activity of aqueous fraction of *Ficus racemosa* leaves extract"** submitted to the Department of Pharmacy, East West University, Dhaka, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by Nipa Rani Devi, ID# 2011-3-70-039 in 2015.

_____

Chairperson

Department of Pharmacy

East West University, Dhaka

ACKNOWLEDGEMENTS

All praise is for Almighty for all the bounties granted to me and only with His guidance and help this achievement has become possible.

It is my pleasure and also privilege to express my heartiest regards and gratitude to my respected teacher and research supervisor **Abdullah-Al-Faysal**, Lecturer, Department of Pharmacy, East West University; for his scholarly guidance, constant supervision and support.

It is also a great pleasure for me to offer my deepest gratitude to **Dr. Chowdhury Faiz Hossain**, Chairperson, Department of Pharmacy, East West University for giving me all the necessary support.

I would also like to extend my thanks to all the research students in the lab, lab officers and other staffs of the Department of Pharmacy for their help and assistance, friendly behavior and earnest co-operation which enabled me to work in a very congenial and comfortable ambience.

I owe special thanks to my fellow research group members for their immense support and contribution in my research work.

Last but not the least, I would like to thank my family, and friends for their care and encouragement during my research work.

Dedication

This Research Paper is dedicated to

My beloved parents,

Who are my biggest inspirations…

ABSTRACT

The purpose of the study was to evaluate the cytotoxic, antimicrobial and antioxidant activity of aqueous fraction of *Ficus racemosa* (Family: Moraceae) leaves extract. The powdered leaves of *Ficus racemosa* were extracted with methanol and then partitioned with petroleum ether, ethyl acetate, DCM and aqueous consecutively. The aqueous fraction was used to evaluate cytotoxic, antimicrobial and antioxidant activities. The cytotoxic activity was measured by brine shrimp lethality bioassay. The antimicrobial activity was assessed by disc diffusion method. Aqueous fraction showed cytotoxic activity with LC_{50} value 13µg/ml in brine shrimp lethality test. In antimicrobial activity investigation the aqueous fraction showed low to moderate antibacterial and antifungal activity against the tested organism compared to the ciprofloxacin (30µg/disc) that was used as positive control. The fraction contained 24.631 mg AAE/g of total phenolic content and 32.941mg AAE/g total flavonoid content. The results of study clearly indicate the presence of cytotoxic, antimicrobial and antioxidant properties of aqueous extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

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

CONTENTS

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE REVIEW

List of contents **Page number**

CHAPTER 3: METHODS & MATERIALS

List of contents **Page number**

CHAPTER 4: RESULTS & DISCUSSION

List of contents **Page number**

CHAPTER 5: CONCLUSION

CHAPTER 6: REFERENCE

LIST OF TABLES

LIST OF FIGURES

Chapter: 1

INTRODUCTION

1.1 Introduction

Earth is a planet dominated by plants. The green plant is fundamental to all other life. The oxygen we bnsthe, the nutrients we consume, the fuels we bum and many of the most important materials we use wen produced by plants. Plants represent the first stage in the evolution of living things. In the process of the growth of nature, plants multiplied in number, variety and types. Human has identified as many as 7.5 lakhs species of plants on earth, of which 5 lakhs are classified as "higher plants" and 2.5 lakhs as "lower plants" (Subraman, 2015) The association between plant and man is an age-old process starting from human civilization. There has always been a race between nature and human knowledge. The plants sustain nature and nature sustains them. The interdependence of man and nature increases day by day. If human race makes sensible use of nature, posterity is bound to be prosperous (Subraman, 2015). A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants” (Samy, 2008). Although, there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties termed as medicinal plants (Samy, 2008). Plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, antidiarrheal as well as various therapeutic activities. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the 49 treatment of various diseases. However, scientific studies have been conducted on only a relatively few medicinal plants, and then only to a superficial extent (Faysal, 2008). Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country (Faysal, 2008). Plants are a source of large amount of

drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties (e.g.; *Penicillium notatum*) in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers (Faysal, 2008).

1.1.1 Definition

The plants having therapeutic or medicinal effects are called medicinal plants. The term 'medicine' can be referred to a preparation or as compound containing one or more drugs or therapeutic agents which are used in the treatment, cure or mitigation of various diseases and external or internal injuries of man and other animals (Ghani, 1998).

Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: " A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesizing of useful drugs" (Sofowara, 1982).

1.1.2 Natural Products in Medicine

Natural product medicines have come from various sources of materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates. The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. The industrial revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use

of natural products has had magical religious significance and different points of view regarding the concepts of health and disease existed within each culture (Farnsworth *et al.*, 1966).

However, the penicillin discovered from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristrine and vinblastine from *Catharan thus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Yusuf *et al.*, 1994).

1.1.3 Medicinal Plants

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug administration or European Food Safety Authority to have medicinal effects (Newman *et al.*, 2003).

World Health Organization (WHO) has provided a definition of medicinal plants, which is "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis". It was reported that 80% of the world's population depends on medicinal plants for their primary health care. This scenario is similar to the one occurring in Bangladesh. Bangladesh is an Asian country where only 20 % of the people can be provided with modern

healthcare services while the rest 80 % are dependent on traditional plant-based systems. In the Plant Kingdom, Medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees (UNDP, 1999).

In a study it has been shown that about 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate with their traditional use. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins (Abayomi, 1993). The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized.

Pharmacology is the study of the therapeutic value and/or potential toxicity of chemical agents on biological systems. It targets every aspect of the mechanisms for the chemical actions of both traditional and novel therapeutic agents. In its entirety, pharmacology embraces knowledge of the sources, chemical properties, biological effects and therapeutic uses of drugs. Pharmacological studies range from those that examine the effects of chemical agents on sub-cellular mechanisms, to those that deal with the potential hazards of pesticides and herbicides, to those that focus on the treatment and prevention of major diseases with drug therapy. Several medicinal plants can be employed to produce extracts exhibiting biological effects. It is estimated that only 500 medicinal plant species had been recorded in Bangladesh out of approximately 1900 species regarded as having medicinal value (Yusuf *et al.*, 1994).

1.1.4 History of Plants in medicine

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The ‘Pen Tsao’ contains thousands of herbal cures attributed to Shen-nung, China’s legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs. Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments. Early Roman

writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in De Materia Medica. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years (Bryan *et al*., 1989).

The beginning of the Renaissance saw a revival of herbalism in the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that is should be used to promote male virility and ensure conception (Ghani, 1998)

Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants. While Western medicine strayed away from herbalism, 75% to 90% of the rural population of the rest world still relies on herbal medicine as their only health care (Levetin and Mahon, 2003).

In many village marketplaces, medicinal herbs are sold alongside vegetables and other wares. The People's Republic of China is the leading country for incorporating traditional herbal

medicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices (Levetin and Mahon, 2003).

1.1.5 Why some of the plants are valued as medicinal plants

Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism). Chemicals that make a plant valuable as medicinal plant are

i. Alkaloids (compounds has addictive or pain killing or poisonous effect and sometimes help in important cures,
ii. Glycosides (use as heart stimulant or drastic purgative or better sexual health),
iii. Tannins (used for gastrointestinal problems like diarrhoea, dysentery, ulcer and for wounds and skin diseases),
iv. Volatile/essential oils (enhance appetite and facilitate digestion or use as antiseptic/insecticide and insect repellent properties),
v. Fixed oils (present in seeds and fruits could diminish gastric/acidity),
vi. Gum-resins and mucilage (possess analgesic property that suppress inflammation and protect affected tissues against further injury and cause mild purgative), and
vii. Vitamins and minerals (Fruits and vegetables are the sources of vitamins and minerals and these are used popularly in herbals) (Ghani A,1998).

1.1.6The importance of medicinal plant in drug discovery

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials.

Globally, there have been concerted efforts to monitor quality and regulate the growing business of phytodrugs and traditional medicine. Data analysis confirms that the phytodrugs serve as crucial sources for the new drugs which can be further modified during drug development.

Studies reveal that the origin of the drugs developed in the last two decades showed that natural products or natural–derived drugs comprised of 28 per cent of all New Chemical Entities (NCEs). Not only were this 24 per cent of the NCEs synthetic or natural base compounds thus elevating the percentage of such products to nearly 52 per cent of all NCEs. This confirms the fact that the natural products including phytodrugs serve as crucial sources for the new drugs which can be further modified during drug development. According to World Health Organization (WHO), as much as 80 per cent of the world’s population relies on traditional medicine. With increased concerns about rising healthcare costs, some governments are encouraging the use of indigenous systems of medicine rather than expensive imported drugs. This has been a strong driver for the resuscitation of phyto products. Today's healthcare systems rely largely on medicinal plant material world's population depends on traditional medicine to meet daily health requirements, especially within developing countries. Use of plant-based remedies is also widespread in many industrialized countries and numerous pharmaceuticals are based on plant compounds. The pharmaceutical industry is both large and highly successful. At present, about 50 per cent of the total plant-derived drug sales come from single entities, while the remaining 50 per cent come from herbal remedies. Although the latter have greater volumes of consumption, the relatively low volumes of single entities, which are mostly prescription products, are more than compensated by their higher prices. Single entity plant drugs, which mostly treat serious medical ill, include atropine, digoxin, morphine, paclitaxel, pilocarpine, reserpine, scopolamine, topotecan and vincristine, among many others. Several of the

compounds have outlived their usefulness in light of better alternatives, however, and are exhibiting decline in sales. On the other hand, as a consequence of new drug developments, single entities overall are projected to increase their market share of the combined total future dollar sales.

1.1.7 Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in "Materia Medica" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants, majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (Kritikar and Basu, 1980).

Bioactive compounds deposited in medicinal plants can serve as important raw materials for pharmaceutical manufacturing. Therefore, well-judged and scientific investigation of this wealth can significantly contribute to the public health. Again, it was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries. Thus, being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries (Chopra *et al*., 1982).

1.1.8 Economic Value

Medicinal plants are good repository of bioactive compounds. They serve as important therapeutic agents as well as essential raw materials for the manufacture of traditional and modern medicines. They, therefore, play a vital role to constitute a precious natural wealth of a country and contribute a great deal to its health care program. A huge amount of foreign exchange can be earned by exporting medicinal plants to other countries. India and Thailand are

two examples of such countries which earn a lot of foreign exchange by exporting medicinal plants and their semi-processed products to other countries including Bangladesh. In this way indigenous medicinal plants take part significantly to build up a healthy economy of a country.

1.2 Research on Herbal Drug

Herbal drug may be defined as the plants, plant parts and plant products of all description, particularly those with medicinal properties. Herbal drugs are generally manufactured by the combination of two or more natural substances. The utility of these combinations are:

- To increase efficacy of the drug.
- To remove toxic effects.
- To reduce side-effects.
- To maintain stability.
- To keep pleasant taste, color and odor.

1.2.1 Scientific Basis of Herbal Drug

Herbal drug is often criticized as non-scientific, inactive and erroneous medicine. But phytochemical and biological investigation proves its medicinal value and therapeutic utility. Traditional medicines that are used topically to treat skin disease contain tannin. Tannin is chemical having antiseptic and astringent property. When it is used topically it reacts with the proteins on infected area to produce a thin but strong barrier. This layer protects the infected area from micro-organism. Besides, tannin has antibiotic property. So it is said that there is no basic difference between herbal drug and allopathic medicine.

1.2.2 Rationale of Herbal Drug Research: Special Reference to Bangladesh

Most of the people of our country have no or little access to allopathic medicine due to their uncompromisable low income in respect of high cost of allopathic medicine. A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% if people suffering illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10%

contact mollahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in a crude and substandard form, by our people. The use of such crude and substandard herbal drug is dangerous and may threaten public health. Thus the analysis of plants for exploring the bounty of chemical entities and their biological screening is the current need for standardization of herbal medication (Ghani, 1998).

Since Bangladesh is a country of low economic growth, a proper health care system can be established by supplying low cost medicines to its population. This may be only possible by utilizing our natural resources of medicinal plants and their constituents. So, scientific exploration and standardization of these potential crude drugs is an urgent need to revolutionize our drug sector.

Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. During the last five years Bangladesh has spent more than 1500 core Taka for importing chemicals, raw materials and semi-processed drugs of plant origin from neighboring and other countries and this trend is growing upwards day by day. This huge foreign exchange can be saved if the indigenous medicinal plants or its semi processed products are utilized by the manufacturer to satisfy their need (Ghani, 1998).

1.3 Natural Sources: A Model for Synthetic Drugs

Natural sources are contributing to the development of modern synthetic drugs and medicines in a number of ways as stated below (Ghani, 1998):

i. Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.
ii. Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.
iii. Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs.

Though most of the modern medicines are gift of synthetic chemistry, there are still some synthetic drugs where plant constituents act as "lead" (precursor) molecule. Procaine, a synthetic compound, displaces cocaine, isolated from coca leaves, due to its lacking of addiction property. Due to relatively low therapeutic index of procaine, search of new synthetic products lead to synthesis of Lidocaine, tetracaine and dibucaine. The discovery of diosgenin from Mexican Yams (Dioscoria) as a starting material for the synthesis of progesterone decreases the cost of progesterone from 80 U.S. $ per gm to 1.7 U.S. $ per gm. Also a life saving antibiotic penicillin is synthesized from a natural product 6-aminopenicillanic acid derived from *Penicillium notatum*(Goldstein *et al*., 1974).

1.3.1 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 (*Vincarosea*) 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*et al*., 1982). Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammivi snaga* which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Ghani, 1998).

1.3.2 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:

i. Selection and correct identification of the proper medicinal plant.
ii. Extraction with suitable solvent(s).
iii. 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.
iv. Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.
v. Repeated fractionation of active fractions to isolate pure compound(s).
vi. Elucidation of chemical structure of pure compound(s) using spectroscopic methods.
vii. Evaluation of biological activity of pure compound(s)
viii. Toxicological tests with pure compound(s).
ix. Production of drug in appropriate dosage forms.

1.3.3 Bioactivity Guided Research of Medicinal Plants

However, natural products are currently undergoing a phase of reduced attention in drug discovery because of the enormous effort which is necessary to isolate the active principles and to elucidate their structures (Grabley and Thiericke, 1999). Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, information from traditional medicine, field observations or even random collection. One main strategy in the isolation of new leads consists of the so-called Bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. Bioactivity guided phytochemical approach, has three phases of investigation.

First, biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.

Second, the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained.

Third, the chemical structures of pure compounds are determined.

Only the bioactive extracts or fractions would be of connotation for next phytochemical and pharmacological analysis. So in medicinal plants research, bioactivity guided phytochemical approach might be a rational approach.

1.4 Description of *Ficus racemosa:*

1.4.1 Scientific name:

Ficus racemosa Linn.

1.4.2 Common names:

Cluster fig, Cluster tree, Country fig, Redwood fig, Juguorong, Crattock, Dumurfikon

1.4.3 Local names:

Jagadumur, Gulangdumur, Yajnadumbar

1.4.4 Taxonomic position:

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Superorder: Urticanae

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: ***F. racemosa***

1.4.5 Description

Leaves are ovate, ovate-lanceolate or elliptic, subacute, entire and petiolate and are shed by December and replenished by January and April, when the tree becomes bare for a short period. It is seen dwelling in areas up to 1200 m altitude on hilltop. This requires well-drained, medium to heavy soils for its successful cultivation and comes up in all kinds of soils except in water logged and clay soil. The plant is propagated by using cuttings of stem and root suckers. Seeds can also be used for propagation. The flowers are pollinated by very small wasps. It has evergreen leaves, if it is close to a water source. Otherwise it sheds its leaves in January. Figs have been traditionally used by children to play. Thin sticks can be joined by inserting them in goolar figs to make interesting shapes; it can grow over 40 feet tall and 20 to 40 feet wide.

(A) (**B)** **(C)**

Figure 1.1: Fruits (A), Bark (B) and Leaves (C) of *Ficus racmosa* Linn

1.4.6 Medicinal uses:

Ficus racemosa Linn has been extensively used in traditional medicine for a wide range of ailments. Its bark, fruits, leaves, roots, latex and seeds are medicinally used in different forms, sometimes in combination other herbs.

1.4.6.1 Bark

Bark is reddish grey or grayish green, soft surface, uneven and often cracked, 0.5-1.8 cm thick, on rubbing white papery flakes come out from the outer surface, inner surface light brown, fracture fibrous, taste mucilaginous without any characteristic odour15, 16. It is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery and piles.

1.4.6.2 Leaves

Leaves are dark green, 7.5-10 cm long, glabrous; receptacles small subglobose or piriform, in large clusters from old nodes of main trunks. The leaves are good wash for wounds and ulcers. They are useful in dysentery and diarrhea. The infusion of bark and leaves is also employed as mouth wash to spongy gums and internally in dysentery, menorrhagia, effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis (Bheemachari*et al.,* 2007).

1.4.6.3 Fruits

The fruits receptacles are 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening. The fruit of *Ficus racemosa* Linn is ¾ inch to 2 inches long, circular and grows directly on the trunk. Tender fruits are astringent, stomachic, refrigent, dry cough, loss of voice, disease of kidney and spleen, astringent to bowel, styptic, tonic, useful in the treatment of leucorrhoea, blood disorder, burning sensation, fatigue, urinary discharges, leprosy, intestinal worms and carminative. They are useful in miscarriage, menorrhagia, spermatorrhoea, cancer, scabies, haemoptysis, and visceral obstructions (Hodge *et al.*, 1998).

1.4.6.4 Roots

The roots of *Ficus racemosa* Linn are long and brownish in colour. It's having characteristic odour and slightly bitter in taste. Roots are used in dysentery, pectoral complaints, and diabetes, applied in mumps, other inflammatory glandular enlargements and hydrophobia (Vedavathy *et al*., 1991)

1.4.6.5 Latex

Latex is aphrodisiac and administered in hemorrhoids, diarrhea, diabetes, boils, traumatic swelling, toothache and vaginal disorders.

1.4.7 Toxicology

As one of the oldest known human foods, figs as a fruit have a very high safety profile. However, the toxicological evaluation of other fig products is in an early stage. Skin contact with latex may provoke allergic reactions like dermatitis, asthma and anaphylaxis (Chelminska, 2004) while orally administered latex may induce hallucinosis (Luna, 1984). Effects other than discussed abovemay are therapeutic or toxic, depending on the clinical context (Ayinde *et al*., 2007).

1.5 Aims of the Present Study

Attempts should be continued for the evaluation of the cytotoxic, antimicrobial, and antioxidant activity of the aqueous fraction of *Ficus racemosa* leaves extract. To conduct cytotoxic investigation of aqueous extract by brine shrimp lethality bioassay. To investigate in vitro antioxidant property of aqueous extract by total Phenolic content and total Flavonoid content. *Ficus racemosa* is a very common plant which is used in our country as well as in world by a lot of people for several purposes. All the parts of this plant are used for medicinal activity. To achieve this objective, the whole work was designed in the following way:

1. Cytotoxic study with aqueous fraction.
2. Observation of antimicrobial action with aqueous fraction.
3. Antioxidant study with aqueous fraction.

Chapter: 2

LITERATURE REVIEW

2.1 Review of literature

2.1.1 Phytochemical Studies

The medicinal plant, *Ficus racemosa* L. Was analyzed for its chemical composition. Chemical analysis showed that the plant is rich in nutrients, especially antioxidant compounds such as total phenol, vitamin C and β-carotene. Phytochemical screening showed that the methanolic extract contains the bioactive constituents such as tannins, saponins, phenols, flavonoids and terpenoids .

F. racemosa has been widely used for its medicinal and pharmaceutical properties throughout the world. The present review is the pool information that highlights the botany, chemical constituents, and pharmacological activities. *F. racemosa* is rich in beta-carotene also it contains a good amount of complex carbohydrates, fiber, and a vast array of vitamins B and minerals. It is also a valuable source of nutrients. The chemical composition and medicinal uses of *F. racemosa* extract have been reported widely. The title plant has been reported to contain racemosic acid, bergenin, tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, ficusin, coumarin and phenolic glycosides. Naghma Khan *et al.*, correlates the presence of lupeol, quercetin and alpha-sitosterol in the ethanol extract of title plant with chemoprevention and decreased oxidative stress in rats. In the present study, we quantified major constituent, gallic acid by HPLC method. Earlier, we also reported the quantification of bergenin, lupeol and alpha-sitosterol in FRE using HPLC and HPTLC methods. The stem bark is antiseptic, antipyretic and used in the treatment of various skin diseases, ulcers, diabetes, piles, dysentery, asthma, gonorrhea, menorrhagia, leucorrhea, hemoptysis and urinary diseases. Fruits are a good remedy for visceral obstruction and also useful in regulating diarrhea and constipation (Kīrtikara *et al.*, 1975). A uterine tonic prepared using the aqueous extract of fruits was found to show effect similar to oxytocin, 4 Antiulcer, hypoglycemic and antioxidant activities from fruits have been reported. Antioxidant, anti-inflammatory, antifungal, analgesic, antipyretic, antibacterial, antidiarrheal, hepatoprotective, hypotensive and various other activities of the leaves have also been evaluated (Forestieri*et al.*, 1996). A glance at literature revealed the isolation of triterpenoids, steroids, coumarins and phenolic esters from fruits, latex, leaves, heartwood and stem bark and only one reference reporting the isolation of alpha-sitosterol from root bark.

2.1.2 Pharmacological Studies

2.1.2.1 Antibacterial Activity

The bioactive compounds of fruits of *Ficus racemosa* were investigated for antibacterial activity against some pathogenic bacteria. The organic extracts (petroleum ether and methanol) showed the highest activity against the test bacteria. The activity was more pronounced on fungi (two species), Gram-positive (three species) and Gram-negative bacteria (five species). This study was identified the antimicrobial activities of the methanol extracts from the stem bark of *Ficusovata*(FOB), fractions (FOB1–6) and compounds isolated following bio-guided fractionation 3-friedelanone , taraxeryl acetate, betulinic acid, oleanoïc acid, 2-hydroxyisoprunetin, 6,7-(2-isopropenyl furo)-5,2,4-trihydroxyisoflavone, Cajanin and protocatechuic acid. The lowest MIC value (156g/ml) observed with the crude extract was recorded on *Streptococcus faecalis, Candida albicans* and *Microsporum audouinii*. The corresponding value for fractions (39mg/ml) was noted with FOB4 against *Staphylococcus aureus*, while that of the tested compounds (10mg/ml) was observed with compound protocatechuic acid on *Microsporum audouinii* (Kuete *et al.,* 2009).

Evaluate the antibacterial activities of various sequential extract of *Ficus racemosa* stem bark. The study evaluated the antibacterial activity of sequential extracts of *Ficus racemosa* stem bark against *Staphylococcus aureus, Bacillus cereus* , *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*by disk-diffusion and agar-diffusion methods. In disk-diffusion assay chloroform, acetone and methanol extracts showed moderate antibacterial against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* compared to the positive control, while petroleum ether extract did not exhibit antibacterial activity against any of the organisms tested. Aqueous extract inhibited only *Bacillus subtilis*, while none of the extracts inhibited *Pseudomonas aeruginosa.* In agar-diffusion assay, both petroleum ether and aqueous extract did not show any inhibitory activity against any of the test organisms, while methanol extract showed moderate activity against *Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli*. Acetone extract showed moderate inhibition of *Staphylococcus aureus, Bacillus cereus* and FRSCE inhibited *Bacillus subtilis* and *Escherichia coli* to some extent (Faiyaz *et al.,* 2010).

Evaluate the antibacterial properties of *F. pseudopalma blanco* crude ethanolic leaf extract, and its solvent fraction chloroform, aqueous and water fraction. All extract exhibited no antibacterial activity agnist Gram-negative bacteria but more potential antibacterial agent against Gram-positive bacteria (Llagas *et al.*, 2014).

All the samples to be activated to *Mycobacterium smegmatis*. The results of the diffusion test indicated that the crude extract were able to prevent the growth of all tested (fungi, Gram-positive and Gram-negative bacteria) organisms (Kuete *et al.*, 2008).

A coarse powder of the leaves of *Ficus racemosa* Linn. was prepared and dried at 500c. The coarse powder extracted using hydro alcohoic (methanol: water) in soxhlet apparatus. The extracts were then subjected to photochemical screening using standard procedure. The extract was screened against bacteria i.e. *Actinomyces Viscosus* (MTCC 7345). Agar Broth Dilution technique and Cup Plate Diffusion Method use to assay. Agar broth dilution technique shows that the lowest concentration that inhibited the growth of microorganism *Actinomyce sviscosus* was 0.08 mg/ml. From this data Minimum Inhibitory Concentration of the extracts of *Ficus racemosa* Linn leaves for microorganism *Actinomyce sviscosus* was found to be 0.08mg/ml. Cup Plate Diffusion Method the concentration of extract of *Ficus racemosa* Linn. leaves 0.08 mg/ml, 0.09 mg/ml; 0.1 mg/ml shown the zone of inhibition. The extract of *Ficus racemosa* Linn leaves of 0.08mg/ml to 0.1mg/ml has better antibacterial activity (Ayinde *et al.*, 2007).

2.1.2.2. Chemomodulatory Activity

Study showed the chemo preventive effect of *Ficus racemosa* extract against Fe-NTA-induced renal oxidative stress, hyper proliferative response and renal carcinogenesis in rats. It also enhances blood urea nitrogen, serum creatinine, ornithine decarboxylase (ODC) activity and thymidine [3H] incorporation into renal DNA. It also enhances DEN (Ndiethylnitrosamine) initiated renal carcinogenesis by increasing the percentage incidence of tumors. Treatment of rats orally with *F. racemosa* extract (200 and 400 mg/kg body weight) resulted in significant decrease in gglutamyl transpeptidase, lipid peroxidation, xanthine oxidase, H_2O_2 generation, blood urea nitrogen, serum creatinine, renal ODC activity, DNA synthesis and incidence of tumors. Renal glutathione content ($P < 0.01$), glutathione metabolizing enzymes and antioxidant enzymes were also recovered to significant level (Khan *et al.*, 2005).

2.1.2.3 Antioxidant activity

The presence of antioxidant compounds, methanol and 70% acetone (acetone: water, 70:30) extracts of *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) were evaluated for their antioxidant activity and radical scavenging capacity in comparison with *Camellia sinensis* (L.) O. Kuntz (green tea). Methanol extracts of green tea and *F. bengalensis* and 70% acetone extract of *F. racemosa* contained relatively higher levels of total phenolics than the other extracts. Though all the extracts exhibited dose dependent reducing power activity, methanol extracts of all the samples were found to have more hydrogen donating ability. All the extracts exhibited antioxidant activity against the linoleic acid emulsion system (34–38%). The potential of multiple antioxidant activity was evident as it possessed antihemolytic activity and metal ion chelating potency (MANIAN *et al.*, 2008).

In the study evaluated of secondary metabolites from *Ficus racemosa* root bark and antioxidant activity of root bark and heartwood. Compounds isolated include one long chain hydrocarbon (n-hexacosane), eight triterpenes (polypodatetraene, a-amyrin acetate, gluanol acetate, lupeol acetate, b-amyrin acetate, 24,25-dihydroparkeol acetate, a-amyrinoctacosanoate including a novel lanostane derivative, lanost-20-en-3b-acetate), an isocoumarin (bergenin) and two phytosteroids (b-sitosterol and b-sitosterol-b-D-glucoside). Significant antioxidant activity was observed. All compounds except b-sitosterol are being reported for the first time from the root bark of this species. Root heartwood was found to be a more effective antioxidant agent (Jain *et al.,* 2013).

Bioactivity guided fractionation study showed antioxidant activity of Ethanolic extract of *Ficus racemosa* stem bark. Study observed antioxidant and renoprotective activity in our study also suggested due to the presence of one or more of these compounds. For the first time, that study evaluated the antioxidant effects of the edible young leaves of *F. virens var. sublanceolata, F. auriculata, F. vasculosa, F. callosa, F. virens var. verins, F. racemosa* and *F. oligodon* along with their total phenolic and flavonoid contents. Which results showed that the edible young leaves of the seven *Ficus* species possess abundant antioxidants at various concentrations, and the ethanol extracts of *F. virens var. sublanceo lata* and *F. auriculata* showed considerable high antioxidant potential compared with other species tested (Veerapur *et al.*, 2011).

Ethanol extract (FRE) and water extract (FRW) of *Ficus racemosa* Linn. were subjected to free radical scavenging both by steady state and time resolved methods such as nanosecond pulse radiolysis and stopped-flow spectro photometric analyses. FRE exhibited significantly higher steady state antioxidant activity than FRW. FRE exhibited concentration dependent DPPH, ABTS, hydroxyl radical and superoxide radical scavenging and inhibition of lipid peroxidation with IC_{50} comparable with tested standard compounds. In vitro radio protective potential of FRE was studied using micronucleus assay in irradiated Chinese hamster lung fibroblast cells (V79). Pretreatment with different doses of FRE 1h prior to 2 Gy γ-radiations resulted in a significant ($P< 0.001$) decrease in the percentage of micro nucleated binuclear V79 cells. Maximum radioprotection was observed at 20 μg/ml of FRE. The radioprotection was found to be significant ($P< 0.01$) when cells were treated with optimum dose of FRE (20 μg/ml) 1 h prior to 0.5, 1,2, 3 and 4 Gy γ-irradiation compared to the respective radiation controls. The cytokinesis-block proliferative index indicated that FRE does not alter radiation induced cell cycle delay. Based on various results it maybe say that the ethanol extracts of *Ficus racemosa* Linn. acts as a potent antioxidant & probable radio protector.

2.1.2.4 Anti-inflammatory Activity:

The anti-inflammatory activity of *Ficus racemosa* extract was evaluated on carrageenin, serotonin, histamine and dextran-induced rat hind paw oedema models. The extract at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models. The extract (400 mg/kg) exhibited maximum anti-inflammatory effect that is 30.4, 32.2, 33.9 and 32.0% at the end of 3 h with carrageenin, serotonin, histamine, dextran-induced rat paw oedema, respectively. In a chronic test the extract (400 mg:kg) showed 41.5% reduction in granuloma weight. The effect produced by the extract was comparable to that of phenyl butazone, a prototype of a non-steroidal anti-inflammatory agent (Mandal *et al.*, 2000).

2.1.2.5 Anti-pyretic activity:

The anti-pyretic effect of a methanol extract of stem bark of *Ficus racemosa* Linn. (MEFR) on normal body temperature and yeast-induced pyrexia in albino rats. A yeast suspension (10 ml/kg body wt.) increased rectal temperature 19 h after subcutaneous injection. The effect extended up

to 5 h after drug administration. The anti-pyretic effect of MEFR was comparable to that of paracetamol (150 mg/kg body wt.), a standard anti-pyretic agent (Bhaskara*et al.*, 2002).

2.1.2.6 Antifertility Activity:

Hydroalcoholic Extract of *Ficus racemosa* L. reduced fertility to 70% within 60 d. Suppression of cauda epididymis sperm count, motility, viability and abnormal morphology was observed. Marked reduction was noted in the weight of reproductive organs and the level of sialic acid in epididymis and fructose in seminal vesicle. Vaginal application of bark extract exhibited 80% vaginal contraceptive efficacy. After cessation of plant extract treatment, the altered parameters recovered after 60 d. Clinical assessment of male antifertility agents should include acceptability, safety and efficacy during and after the treatment (Ahirwar *et al.,* 2011).

2.1.2.7Antidiabetic Activity:

The present study was designed to investigate the effects of the ethanol extract of *Ficus racemosa* (FRE) stem bark on biochemical parameters in type 2-like diabetes, induced by a combination of standardized high-fat diet and low-dose streptozotocin (25 mg kg1, i.p.) in rats. To elucidate the mode of action of FRE, its effects on a battery of targets involved in glucose homeostasis was evaluated. FRE, in a dose-dependent manner, altered the biochemical parameters and significantly improved glucose tolerance and HDL-c levels. This is the first report demonstrating the effectiveness of *F. racemosa* stem bark in type 2 diabetes and targets involved in it (Veerapur *et al.,* 2012).

Bioactivity\guided fractionation led to the isolation of both vitexin and isovitexin. Oral administration of1mg/kg of either rvitexin or isovitexin significantly reduced the postprandial blood glucose level in sucrose loaded normoglycemic mice at 30min. The percentage of postprandial blood glucose reduction was highest in sucrose loaded induced diabetic rats administered orally with 200mg/kg. Both vitexin and isovitexin did not exertany signs of toxicity at the highest dose of 2g/kg. Both the C-glycosyl bioflavonoids, namely, vitexin and isovitexin exhibited in vivo a-glucosidase inhibition (Choo *et al.,* 2012).

2.1.2.8 Protective effect of tannins from *Ficus racemosa* in hypercholesterolemia and diabetes induced vascular tissue damage:

Evaluate the protective effect of tannins from *Ficus racemosa* on the lipid profile and antioxidant parameters in high meal and streptozotocin induced hypercholesterolemia associated diabetes model rats. The results of this study show that two different doses of tannin supplementation had a favorable effect on plasma glucose and lipid profile concentrations. It also had an influence on attenuating oxidative stress in diabetic rats (Velayutham *et al.,* 2012).

2.1.2.9 Wound healing Activity:

This study was established the wound healing activity of aqueous and ethanolic extract of roots of *Ficus racemosa* (Murti *et al.,* 2012).

2.1.2.10 An unusual thermostable aspartic protease:

The most extensively studied ficins have been isolated from the latex of *Ficus glabrata* and *ficus carica*. However the proteases (ficins) from other species are less known. The purification and characterization of a protease from the latex of *Ficus racemosa* is reported. The enzyme exhibited a broad spectrum of pH optima between pH 4.5–6.5 and showed maximum activity at 60 ± 0.5 C. The enzyme activity was completely inhibited by pepstatin-A indicating that the purified enzyme is an aspartic protease. Its enzymatic specificity studied using oxidized B chain of insulin indicates that the protease preferably hydrolyzed peptide bonds C-terminal to glutamate, leucine and phenylalanine (at P1 position). The broad specificity, pH optima and elevated thermal stability indicate the protease is distinct from other known ficins and would find applications in many sectors for its unique properties (Devaraj *et al.,* 2008).

Chapter: 3

METHODS & MATERIALS

3.1 Collection & Preparation of Plant Material

Plant sample (Leaves) of *Ficus racemosa* was collected from, Gopalgonj in 2014. 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 650gm 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 39^0C with a Heidolph rotary evaporation.

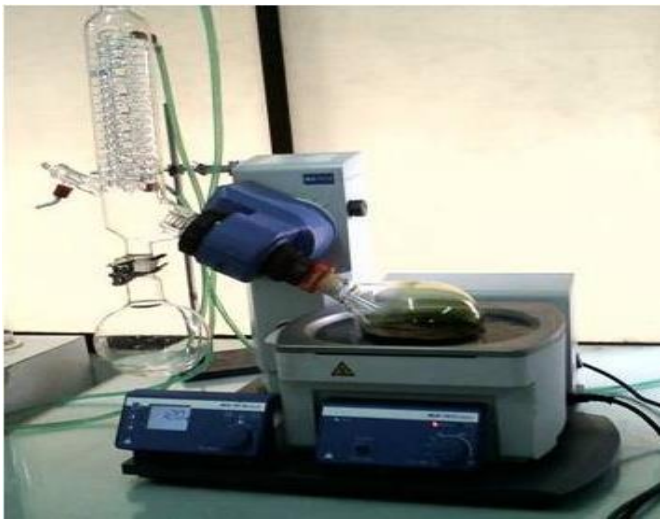


Figure 3.1: Drying of extract using rotary evaporator.

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

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

3.4 Partition of Mother Solution

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

3.4.1 Partition with petroleum ether

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

3.4.2 Partition with DCM

To the mother solution left after partitioning with petroleum ether, 12.5ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with DCM. The process was repeated thrice (100ml 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 partitioning with petroleum ether and DCM, 16ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with ethyl acetate (100ml X 3). The ethyl acetate soluble fractions were collected together and air dried.

3.4.4. Partition with Aqueous

To the mother solution that left after washing with petroleum ether, DCM and ethyl acetate, was then taken in a separating funnel and extracted with Aqueous (100ml X 3). The Aqueous soluble fractions were collected together and air dried.

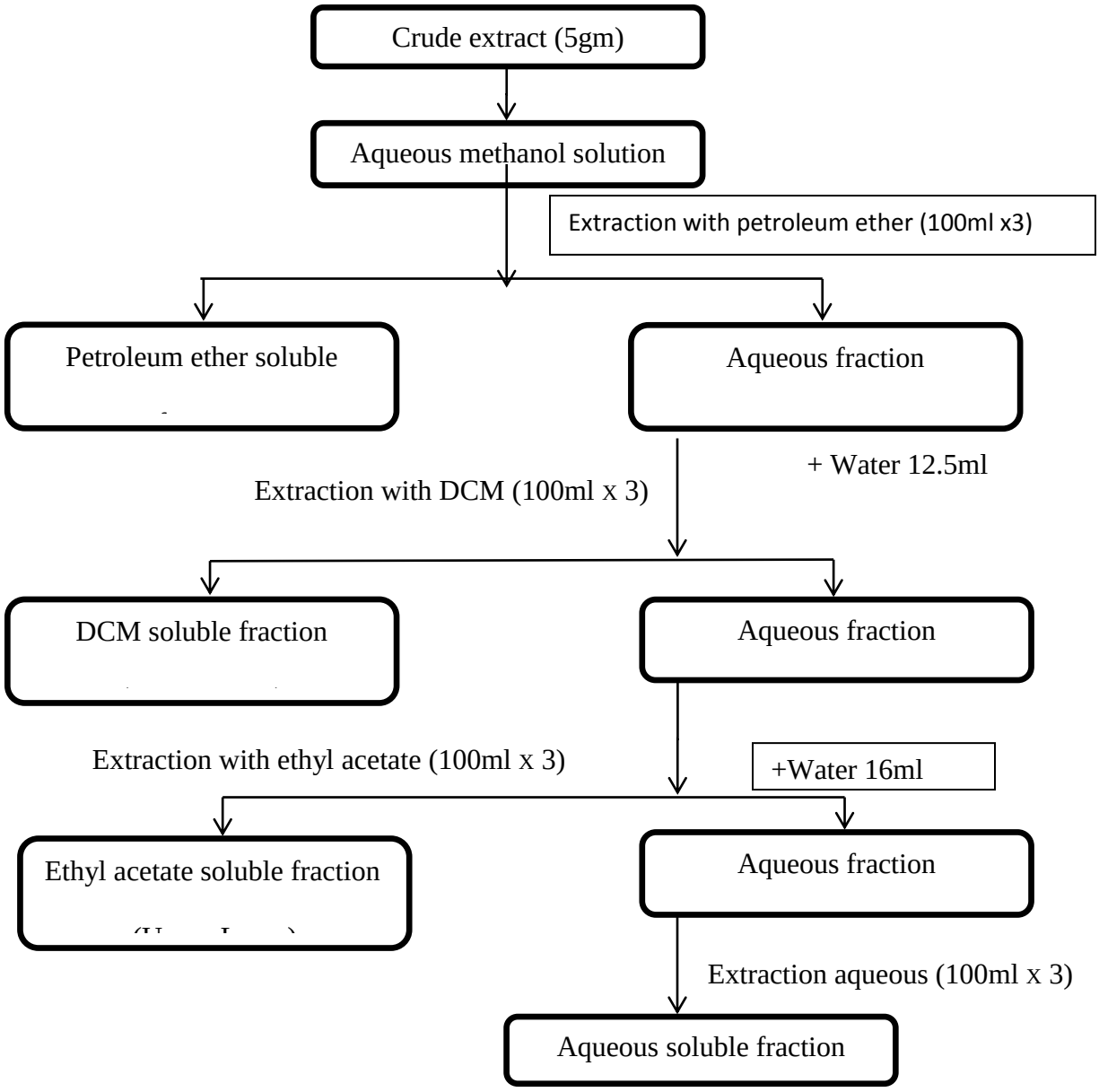


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Ficus racemosa* leaves

3.4.5 Collection of Aqueous Fraction

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

3.5 Brine Shrimp Lethality Bioassay

3.5.1 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (*in-vivo*) lethality, a simple zoological organism, (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested 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).

3.5.2 Apparatus & Reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

Artemia salina leach (brine shrimp eggs)	Pipettes & Micropipette
Sea salt (NaCl)	Glass vials
Small tank with perforated dividing dam to hatch the shrimp	Magnifying glass
Lamp to attract shrimps	Test samples

3.5.3 Procedure

3.5.3.1 Preparation of Sea Water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 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 *Artemia salina* hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the pH 8.4 as sea water.

3.5.3.2 Hatching of Brine Shrimp

A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Then a dry preserved egg of *Artemia salina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemia salina* 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.

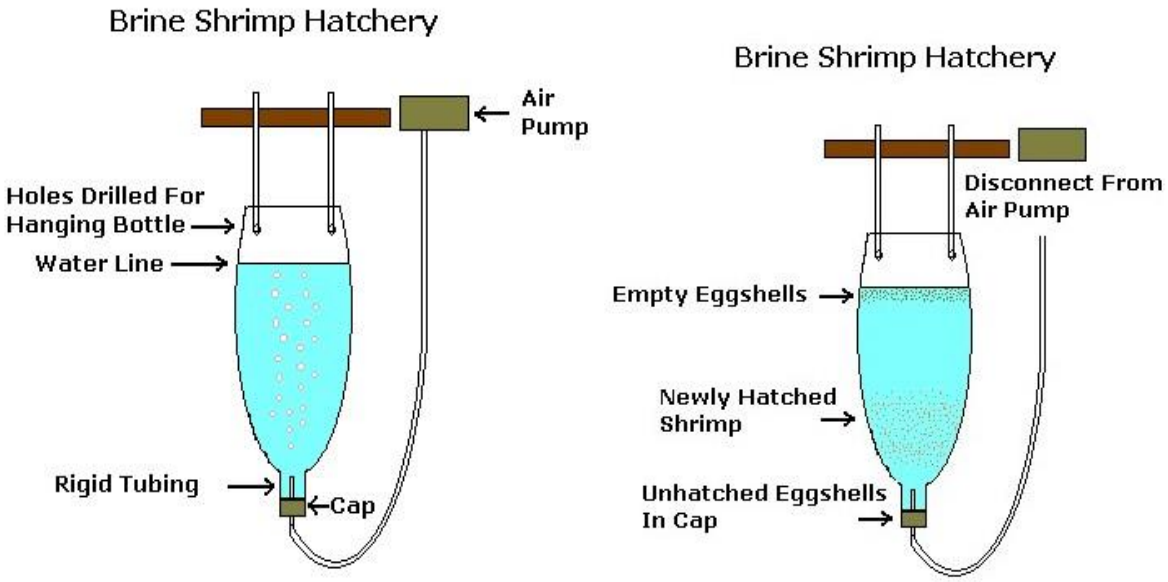


Figure 3.3: Brine shrimp Hatchery

3.5.3.3 Preparation of Test Solutions

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

3.5.3.4 Preparation of the Test Samples of Experimental Plant

All the test samples of 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.

3.5.3.5 Preparation of the Positive Control Group

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 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.

3.5.3.6 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.

3.5.3.7 Counting of Nauplii

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

3.6 Antimicrobial test:

Worldwide, infectious disease is one of main causes of death accounting for approximately one half of all deaths in tropical countries. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods.

• Disc diffusion method

• Serial dilution method

• Bioautographic method

3.6.1 Antimicrobial Activity by Disc Diffusion Method

3.6.1.1 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).

3.6.1.2 Apparatus &Reagents

Table 3.2: 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

3.6.1.3 Test Sample of *Ficus racemosa*

Aqueous fraction of methanolic extract of *Ficus racemosa* leaves were taken as test sample.

3.6.1.4 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 3.3: List of micro-organisms

Type of Bacteria	**Name of Bacteria**
Gram +ve	*Bcillus subtilis* *Bacillus cereus* *Bacillus megaterium* *Streptococcus aureaus*

Gram –ve	*Escherichia coli* *Salmonella paratyphi* *Salmonella typhi* *Pseudomonas aureaus*
Fungi	*Saccharomyces cerevisiae*

3.6.2 Procedure

3.6.2.1 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 3.4: Autoclave machine

3.6.2.2 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 121^0C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

3.6.2.3 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 petridish with the help of this cotton bud.

3.6.2.4 Preparation of Discs

Three types of discs were used for antimicrobial screening.

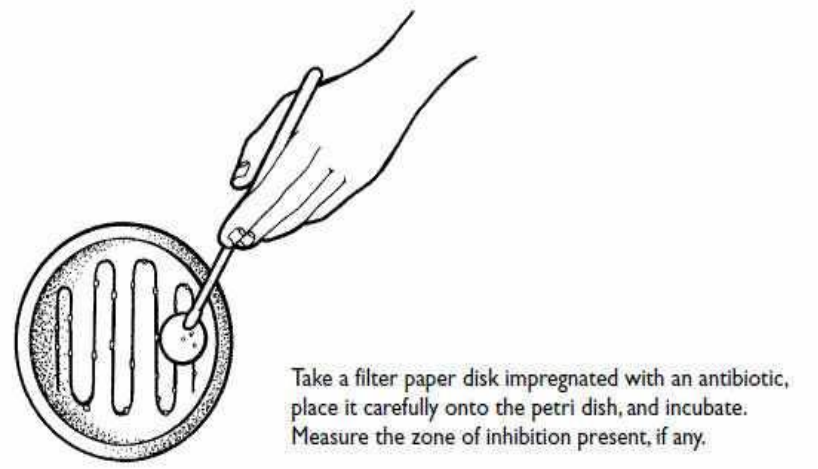


Figure 3.5: Preparation of filter paper discs

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

3.6.2.5 Preparation of Test Sample

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

3.6.2.6 Application of Test Samples

Standard ciprofloxacin 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 air-drying) and the filter paper were not active themselves.

3.6.2.7 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 3.6: Incubator

3.6.2.8 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.

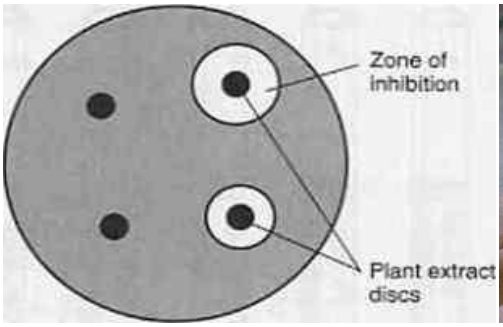


Figure 3.7: Clear zone of inhibition

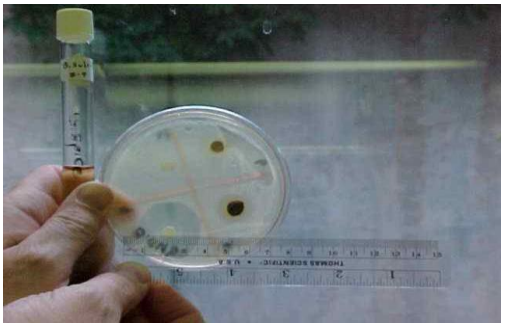


Figure 3.8: Determination of clear zone of inhibition

3.7 Antioxidant Activity

3.7.1 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. 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 *Ficus racemosa*-as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity.

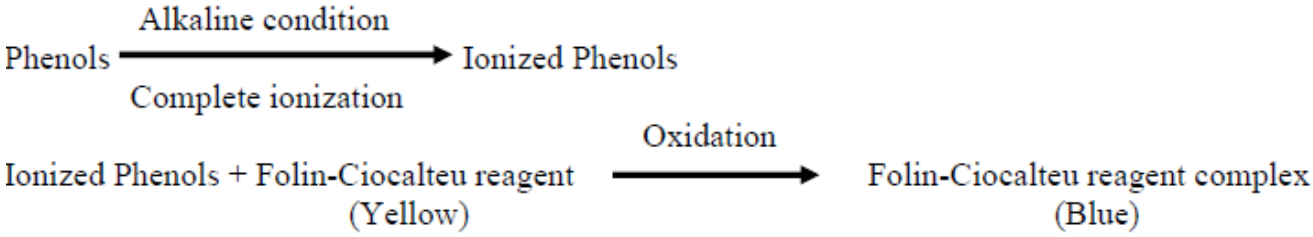
3.7.1.1 Principle

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 3.4: Composition of 100mg Folin-Ciocalteu Reagent

Ingredient	**Amount**
Water	57.5ml
Lithium Sulfate	15.0mg
Sodium Tungstate Dihydrate	10.0mg
Hydrochloric Acid>=25%	10.0mg
Phosphoric Acid 85% solution in water	5.0mg
Molybdic Acid Sodium Dihydrate	2.5mg

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 one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton *et al.*, 1999; Vinson *et al.*, 2005).



3.7.1.2 Apparatus & Reagents

Table 3.5: Apparatus and reagents used for total phenolic content

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

3.7.1.3 Procedure

Standard curve preparation: Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120µg/ml to 80µg/ml. 5ml of FCR (diluted 10 times with water) and4ml of Na_2CO_3 (7.5%w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765nm. 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: 2mg of the *Ficus racemosa* aqueous fraction was taken and dissolved in 1ml of distilled water to get a sample concentration of 2mg/ml.

Determination of total phenol content:

- 1.0ml of plant extract (200µg/ml) of different concentrations (120µg/ml, 110µg/ml, 100µg/ml, 90µg/ml and 80µg/ml) was taken in test tubes.
- 5ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
- 4ml of Sodium carbonate solution was added into the test tube.
- The test tubes containing the samples were incubated for 1hr at the room temperature to complete the reaction.
- Then the absorbance of the solution was measured at 765nm using a spectrophotometer against blank.
- A typical blank solution containing methanol was taken.

3.7.2 Total Flavonoid Content

3.7.2.1 Principle

Aluminium chloride ($AlCl_3$) (Chang *et al.*, 2002) 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 Aor B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorptivity maximum at 510nm (Chang *et al.*, 2002). Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard (Chang *et al.*, 2002).

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

3.7.3.2 Apparatus & Reagents

Table 3.6: 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

3.7.3.3 Procedure

Preparation of 10% Aluminium Chloride ($AlCl_3$) Solution: 10mg of $AlCl_3$ was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

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

Preparation of 5% (W/V) $NaNO_2$ Solution: 5mg of $NaNO_2$ was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of Standard Solution: The stock solution was prepared by taking 0.025gm of ascorbic acid and dissolved into 5ml of ethanol. The concentration of this solution was 5µg/µl of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.7: Preparation of standard solution

Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by ethanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

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

1.5ml extract was taken in a test tube and then 6ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for 6 minutes. 4% NaOH and 0.6ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5ml methanol was taken and the same procedure was repeated. Then the absorbance of the solution was measured at 510nm using a spectrophotometer against blank.

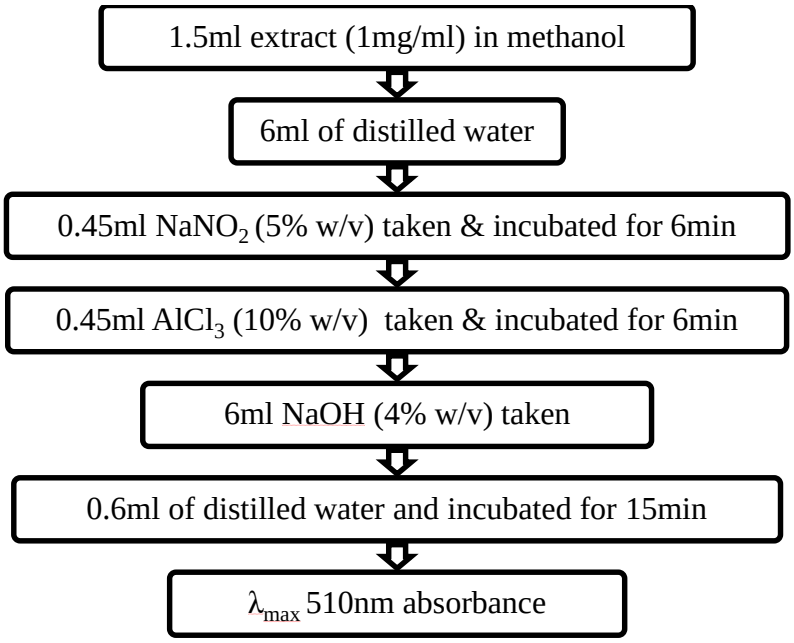


Figure 3.9: Schematic diagram of preparation of extract solution

Preparation of blank solution:

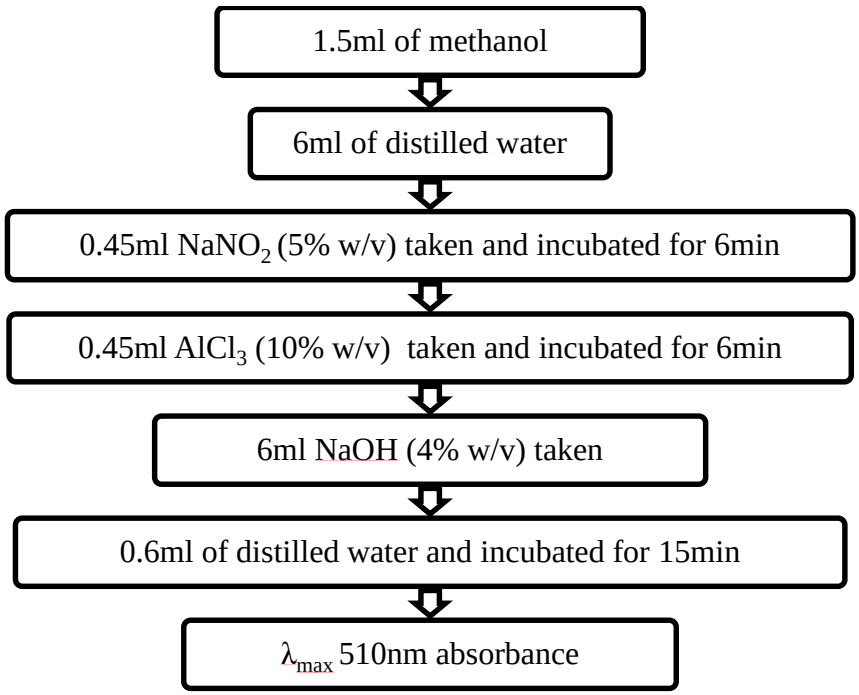


Figure 3.10: Schematic diagram of preparation of blank solution

Chapter: 4

RESULTS & DISCUSSION

4.1 Result of Brine Shrimp Lethality Bio-Assay

The aqueous fraction of the *Ficus racemosa* leaves extract was subjected to brine shrimp lethality bioassay following the procedure. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a median Lethal Concentration (LC_{50}) value. This represents the concentration of the standard or aqueous 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.

4.1.1 Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard

Table 4.1: Results of the bioassay of Tamoxifen (standard)

Test tube no.	**Concentration (C) (µg/ml)**	**LogC**	**Number of nauplii alive**	**Number of nauplii dead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	0	10	100	13.38
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	
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	

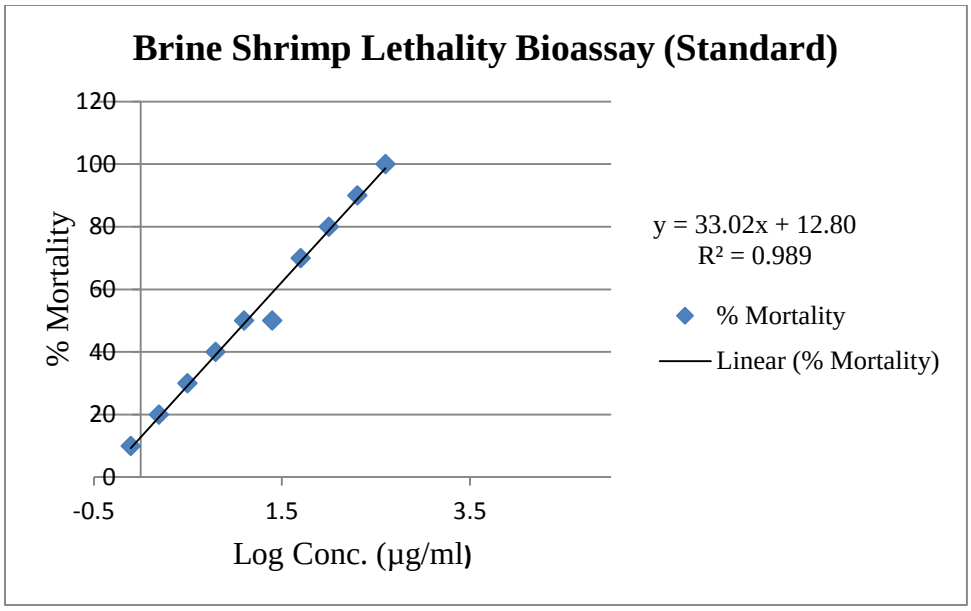


Figure 4.1: % mortality and predicted regression line of Tamoxifen (standard)

4.1.2 Preparation of Aqueous Fraction Curve

Table 4.2: Results of the bioassay of aqueous fraction (extract)

Test tube no.	**Concentration (C) (µg/ml)**	**LogC**	**Number of nauplii alive**	**Number of nauplii dead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	2	8	80	16
2	200	2.301	3	7	70	
3	100	2.000	3	7	70	
4	50	1.699	4	6	60	
5	25	1.398	4	6	60	
6	12.5	1.097	5	5	50	
7	6.25	0.796	5	5	50	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	0.78125	-0.107	8	2	20	

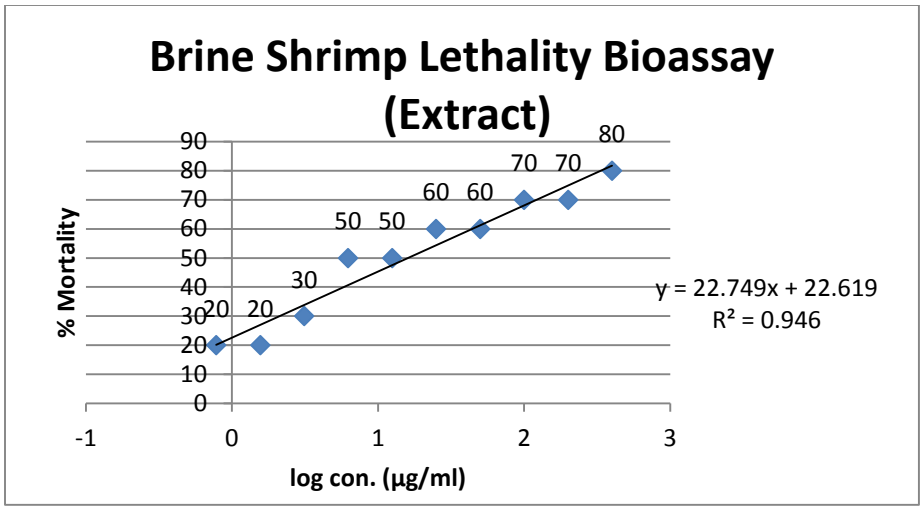


Figure 4.2: % mortality and predicted regression line of aqueous fraction (extract)

4.1.3 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 ranging from the lowest concentration to the highest concentration in both standard and aqueous fraction samples. Mortality increased gradually with an increase in concentration of the test samples. Maximum mortalities took place at the highest concentration of 400µg/ml, whereas the least mortalities at lowest concentration 0.78125µg/ml as shown in Table 4.1 and Table 4.2.

Table 4.3: Cytotoxic activity of Tamoxifen and aqueous fraction of *Ficus racemesa* leaves

Sample	**Linear regression equation**	**R^2 value**	**LC_{50} (µg/ml, 24hr)**
Standard (Tamoxifen)	y = 33.021x + 12.806	0.989	13.38
Extract (Aqueous fraction)	y = 22.749x + 22.619	0.946	16

In this investigation, standard and aqueous fraction exhibited cytotoxic activities with the LC_{50} values 13.38µg/ml and 23µg/ml respectively as shown in Table 4.10. For aqueous fraction R^2 value is less than the standard which indicates that the extract has less potent activity than standard against brine shrimp nauplii.

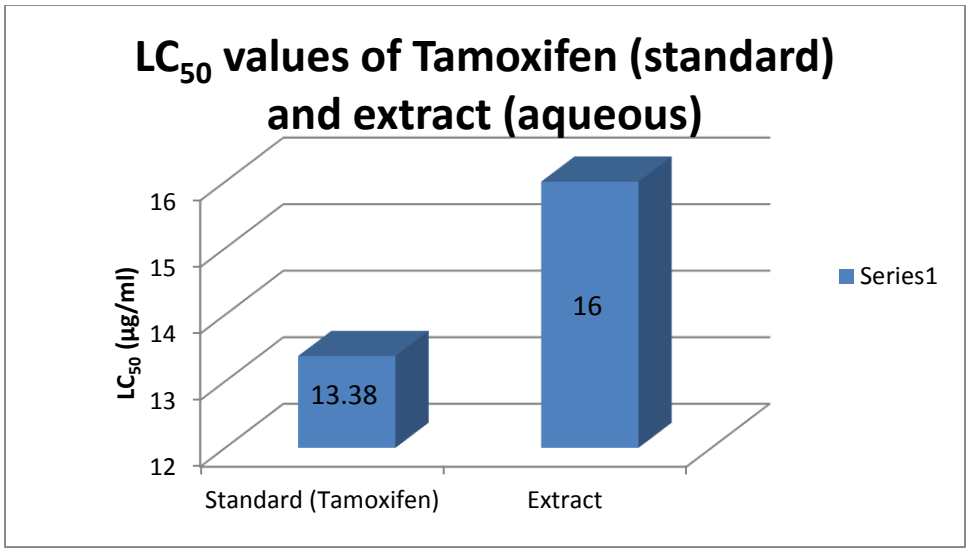


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for aqueous fraction the lethal concentration required to kill 50% of the sample population is higher than the standard. So the extract is less potent than Tamoxifen (Standard) at lower concentration.

4.2 Antioxidant Test Results

Antioxidant tests are classified by various methods. Samples were subjected to various standard methods to determine various scavenging capacity and amount that is equivalent to the standard like ascorbic acids. Antioxidant property of the aqueous fraction of *Ficus racemesa* leaves extract was determined by following methods-

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

4.2.1 Result of Total Phenolic Content

The aqueous extract of leaves and the aqueous fractions of the ethanol extract of *Ficus racemosa* leaves were subjected to determine total phenolic content. Ascorbic acid was used as reference standard.

4.2.1.1 Preparation of Standard Curve

Table 4.4: 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 4.8. This linear curve was considered as a standard curve.

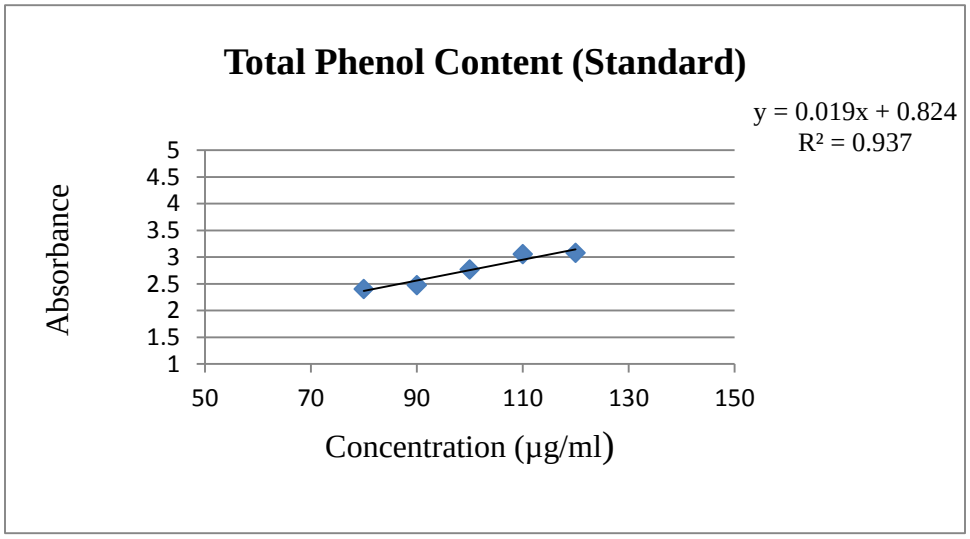


Figure 4.4: Graphical representation of assay of phenolic content of ascorbic acid

4.2.1.2 Total Phenol Content Present in Extract

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 4.5: Total phenolic content of aqueous fraction of leaves of *Ficus racemosa*

Concentration (mg/ml)	**Absorbance**	**X value(mg of AAE/gm of dried extract)**
2	1.292	24.631

4.2.1.3 Discussion

To determine the total phenolic content of the test samples the standard curve was used. For 2mg/ml concentration of aqueous fraction of *Ficus racemosa* (leaves)24.639 mg of AAE/gm of dried extract of phenolic content was found. So it can be said that, the extract contains antioxidative compounds.

4.2.2 Result of Total Flavonoid Content

The aqueous fractions of *Ficus racemosa leaves* were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard.

4.2.2.1 Preparation of Standard Curve

Table 4.6: Total flavonoid content of ascorbic acid

Concentration (µg/µl)	**Absorbance (At 510 nm)**	**Regression line**	**R^2 value**
50	0.05	y = 0.0017x-0.042	0.991
100	0.13		
150	0.19		
200	0.29		
250	0.39		

After absorbance were taken of different solution of ascorbic acid of concentrations ranging from 50µg/µl to 250µg/µl, a linear relationship was observed when the absorbance were plotted against concentrations, as shown in Figure 4.10 This linear curve was considered as a standard curve.

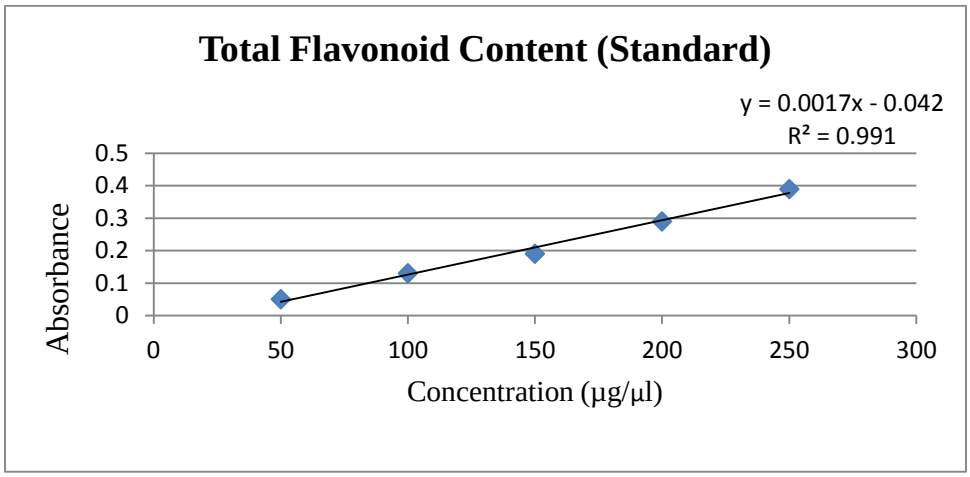


Figure 4.5: Graphical representation of assay of flavonoid content of ascorbic acid

4.2.2.2 Total Flavonoid Content Present in Extract

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 4.11.

Table 4.7: Total flavonoid content of aqueous fraction of *Ficus racemosa leaves* extract

Sample	**Concentration (mg/ml)**	**Absorbance**	**Total flavonoid content (mg of AAE/g of dried extract)**
Aqueous fraction of *Ficus racemosa*	1	0.014	32.941

4.2.2.3 Discussion

To determine the total flavonoid content of the test samples the standard curve was used. For 1mg/ml concentration of aqueous fraction of *Ficus racemosa* (leaves)32.941 mg of AAE/gm of dried extract of flavonoid content was found. So it can be said that, the extract contains antioxidative compounds.

4.3Antimicrobial test results

The antimicrobial activities of aqueous extract of *Ficus racemosa leaves* were examined in the study against various Gram positive bacteria, Gram negative bacteria and fungi. The aqueous fraction was subjected to the various bacterial and fungal cultures and from that zones of inhibition were measured. Here ciprofloxacin was used as standard reference.

4.3.1 Zone of inhibition of standard and aqueous extract

Table4.8: Antimicrobial activity of standard sample (Ciprofloxacin) and aqueous extract.

Type of microorganism		**Zone of inhibition(mm)**	
		Standard sample	**Aqueous extract**
Gram positive bacteria	*Bacillus cereus*	38	10
	Bacillus magaterium	38	9
	Bacillus subtilis	40	9
	Staphylococcus aureus	40	8
Gram negative bacteria control	*Salmonella paratyphi*	38	8
	Salmonella typhi	36	8
	Pseudomonas aureginosa	38	0
	Escherichia coli	36	6
Fungi	*Saccharomyces cerevisiae*	35	6

4.3.2 Discussion

Aqueous extract of *Ficus racemosa* showed moderate to low antimicrobial activity when compared to ciprofloxacin. None of the zone of inhibition of aqueous fraction is equal to ciprofloxacin against any bacteria or fungi. Among all the microbiological cultures, the fraction showed the best antimicrobial activity against *Bacillus cereus* comparable to the standard (30mm).

Chapter: 5

CONCLUSION

Conclusion:

"In All things there is a poison, and there is nothing without a poison. It depends on only upon the dose whether a poison is a poisonor not"-Paracelsus. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The building blocks of plants are explored by researchers which enable to identify elements which can be used in beneficial ways. Plant compounds are playing an important role in many medicines as being the major active compound. As the literature review suggests, the presence of several phytochemical compounds in *Ficus racemosa* makes the plant pharmacologically active. The present study showed that it has moderate antioxidant activity. The aqueous extract possesses cytotoxic activity that could be a better treatment in tumor as well as cancer. The study also showed that, the extract showed moderate antimicrobial activity. 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: 6

REFERENCE

Reference

Abayomi, S (1993), *Historical review of traditional medicine in Africa*, Africa: Spectrum Book ltd., pp. 9-25.

Ahirwar, D., Ahirwar, B. and Kharya, M. (2011).Reversible Antifertility Activity of Hydroalcoholic Extract of Ficus Racemosa L. in Male Mice.*Journal of Reproduction and Contraception*, 22(1), pp.37-44.

Ayinde, B., Omogbai, E. and Amaechina, F. (2007).Pharmacognosy and hypotensive evaluation of FicusexasperataVahl (Moraceae) leaf.*Act a Poloniae Pharmaceutica*, 64, pp.543-546.

Barry, A. L. (1976), *Principle & practice of Microbiology*, 3rd ed. Philadelphia: Lea & Fabager, pp. 21-25.

Bhaskara Rao, R., Anupama, K., Anand Swaroop, K., Murugesan, T., Pal, M. and Mandal, S. (2002). Evaluation of anti-pyretic potential of Ficus racemosa bark.*Phytomedicine*, 9(8), pp.731-733.

Bheemachari, J., Ashok, K., Joshi, N., Suresh, D. and Gupta, V. (2007).Antidiarrhoeal evaluation of Ficus racemosaLinn.latex.*ActaPharmaceuticaSciencia*, 49, pp.133-138.

Bryan, G., Reuden, Harloda, A. (1989), *Wittcoff Pharmaceutical Chemicals in Perspective*, London: South Bank Polytechnic, p. 106.

Chang,C., Yang, M., Wen, H. and Chern, J. (2002) 'Estimations of total flavonoid content in propolis by two complementary colorimetric methods', *Journal of Food and Drug Analysis*, 10: 178-182.

Choo, C., Sulong, N., Man, F. and Wong, T. (2012). Vitexin and isovitexin from the Leaves of Ficus deltoidea with in-vivo α-glucosidase inhibition.*Journal of Ethnopharmacology*, 142(3), pp.776-781.

Chopra, R. N., Chopra, I. C., Hunda, K. I. and Kapur, L. D. (1982), *Chopra's Indigenous Drugs of India*, India: Academic publishers, pp. 1-15.

Devaraj, K., Gowda, L. and Prakash, V. (2008).An unusual thermostable aspartic protease from the latex of Ficus racemosa (L.).*Phytochemistry*, 69(3), pp.647-655.

Faiyaz, A., Sharanappa, P. and Asna, U. (2010). Antibacterial activities of various sequential extracts of Ficus racemosa stem bark. *Pharmacognosy Journal*, 2(8), pp.203-206.

Farnsworth, N. R. (1966) 'Sleeping Giant of Drug Development & Screening of plant', *Journal of Pha* Yusuf, M., Chowdhury, U., Wahab, A., Begum J. (1994) 'Medicinal plant of Bangladesh', *Bangladesh Council of Scientific and Industrial Research* (BCSIR),p. 34.

Faysal, M. (2008).Justification of use of some Medicinal Plants to Treat Various Diseases in Khulna.*Bangladesh.Journal of Ethnobotanical Leaflets*, (12), pp.1231-1235

Forestieri, A., Monforte, M., Ragusa, S., Trovato, A. and Iauk, L. (1996). Antiinflammatory, Analgesic and Antipyretic Activity in Rodents of Plant Extracts used in African Medicine. *Phytotherapy Research*, 10(2), pp.100-106.

Ghani, A. (1998), *Medicinal Plants of Bangladesh*, 1st ed., Dhaka:Asiatic society, pp. 11-41.

Goldstein, A., Aronow, L. and Kalma, S. M. (1974), *Principles of drug action- the basis of pharmacology*, 2nd ed., New York: John Wiley & Sons Ltd., pp. 729-750.

Grabley, S., Thiericke, R. (1999) 'Bioactive agents and natural sources: trends in discovery and application', *Adv Biochem Eng Biotechnol*, 64: 101–154.

Hodge, W. (1998). Book Review:Glossary of Indian Medicinal Plants. R. N. Chopra, S. L. Nayar, I. C. Chopra. *The Quarterly Review of Biology*, 33(2), p.156.

Jain, R., Rawat, S. and Jain, S. (2013). Phytochemicals and antioxidant evaluation of Ficus racemosa root bark. *Journal of Pharmacy Research*, 6(6), pp.615-619.

Khan, N. and Sultana, S. (2005). Chemomodulatory effect of Ficus racemosa extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. *Life Sciences*, 77(11), pp.1194-1210.

Kīrtikara, K., Basu, B. and Blatter, E. (1975).*Indian medicinal plants*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Kritikar, K. R. & Basu, B.D. (1980), *Indian Medicinal Plants*, 2nd ed., India: B. Sing and M. P. Sing, p. 420.

Kuete, V., Nana, F., Ngameni, B., Mbaveng, A., Keumedjio, F. and Ngadjui, B. (2009).Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae).*Journal of Ethnopharmacology*, 124(3), pp.556-561.

Kuete, V., Ngameni, B., Simo, C., Tankeu, R., Ngadjui, B., Meyer, J., Lall, N. and Kuiate, J. (2008). Antimicrobial activity of the crude extracts and compounds from Ficus chlamydocarpa and Ficus cordata (Moraceae). *Journal of Ethnopharmacology*, 120(1), pp.17-24.

Levetin, E., and Mahon, M. (2003), *Plants and Society*, 3rd ed., New York: McGraw-Hill, p. 300.

Llagas, M., Santiago, L. and Ramos, J. (2014). Antibacterial activity of crude ethanolic extract and solvent fractions of Ficus pseudopalma Blanco leaves. *Asian Pacific Journal of Tropical Disease*, 4(5), pp.367-371.

Mandal, S., Maity, T., Das, J., Saba, B. and Pal, M. (2000).Anti-inflammatory evaluation of Ficus racemosa Linn.leaf extract. *Journal of Ethnopharmacology*, 72(1-2), pp.87-92.

MANIAN, R., ANUSUYA, N., SIDDHURAJU, P. and MANIAN, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of Camellia sinensis (L.) O. Kuntz, Ficus bengalensis L. and Ficus racemosa L. *Food Chemistry*, 107(3), pp.1000-1007.

Marcy, J., Balunas, A. and Douglas, K. (2005).Drug discovery from medicinal plants.*Life Sciences*, 78(2), pp.431-441.

Murti, K. and Kumar, U. (2012).Enhancement of wound healing with roots of Ficus racemosa L. in albino rats.*Asian Pacific Journal of Tropical Biomedicine*, 2(4), pp.276-280.

Newman, D. J., Cragg, G. M., Snader, K. M. (2003) 'Natural products of new drugs over the period', *PubMed Central*,66: 1022–1037.

Olowa, L. F. &Nuneza, O. M. (2013) 'Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines', *International Research Journal of Biological Sciences*, 2 (11): 74-77.

Phromthep, W. (2012).A new genetic analysis of Ficus spp. By HAT-Random amplified Polymorphic DNA Technique. *Procedia Engineering*, 32, pp.1073-1079.

Rishikesh, Rahman, M. M., Goffar, Md. R., Mamun, M. R. A., Dutta, P. R. &Maruf, M. A. A. (2013) 'Phytochemical and Pharmacological Investigation of *AchyranthesAspera* Linn', *Scholars Academic Journal of Pharmacy*, 2(2): 74-80.

Samy, R., Pushparaj, P. and Gopalakrishnakone, P. (2008).A compilation of bioactive compounds from Ayurveda.*Bioinformation*, 3(3), pp.100-110.

Sofowora, A. (1982), *Medicinal Plants and Traditional Medicine in Africa*, Africa: John Wiley & Sons Ltd., pp. 6,10,11,74,114,256.

Subraman, K. (2015).An introduction to phytochemical methodology with special reference to flavonoids.*Journal of Indian phytochemicals*, 10, pp.97-102.

UNDP Team (1999),*United Nations Human Development Report*, USA: Oxford University Press, pp. 57-72.

Vedavathy, S. and Rao, K. (1991).Antipyretic activity of six indigenous medicinal plants of Tirumala Hills, Andhra Pradesh, India.*Journal of Ethnopharmacology*, 33(1-2), pp.193-196.

Veerapur, V., Prabhakar, K., Thippeswamy, B., Bansal, P., Srinivasan, K. and Unnikrishnan, M. (2012).Antidiabetic effect of Ficus racemosa Linn.stem bark in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats: A mechanistic study. *Food Chemistry*, 132(1), pp.186-193.

Veerapur, V., Thippeswamy, B., Prabhakar, K., Nagakannan, P., Shivasharan, B., Bansal, P., Sneha, S., Mishra, B., Priyadarsini, K. and Unnikrishnan, M. (2011). Antioxidant and

renoprotective activities of Ficus racemosa Linn.stem bark: Bioactivity guided fractionation study. *Biomedicine & Preventive Nutrition*, 1(4), pp.273-281.

Velayutham, R., Sankaradoss, N. and Ahamed, K. (2012). Protective effect of tannins from Ficus racemosa in hypercholesterolemia and diabetes induced vascular tissue damage in rats. *Asian Pacific Journal of Tropical Medicine*, 5(5), pp.367-373.

Yusuf, M., Chowdhury, U., Wahab, A., Begum J. (1994) 'Medicinal plant of Bangladesh', *Bangladesh Council of Scientific and Industrial Research* (BCSIR),p. 34.