Cytotoxic, antimicrobial and antioxidant activity of crude fraction of *Ficus resemosa* leaves extract

Submitted by:

Nurul Mohammad Shayed

ID: 2011-1-70-003

Research Supervisor

Abdullah-Al-Faysal

Lecturer

Department of Pharmacy

East West University

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

DECLARATION BY THE RESEARCH CANDIDATE

I, Nurul Mohammad Shayed, hereby declare that the dissertation entitled "Cytotoxic, antimicrobial and antioxidant activity of crude fraction of *Ficus resemosa* leaves extract*"***,** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy (Honors) is a legitimate record of original research work carried out by me under the supervision and guidance of **Abdullah-Al-Faysal**, Lecturer, Dept. of Pharmacy and, East West University.

Nurul Mohammad Shayed
ID: 201-1-70-003
Department of Pharmacy
East West University
Dhaka, Bangladesh

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation entitled "Cytotoxic, antimicrobial and antioxidant activity of crude fraction of *Ficus resemosa* leaves extract*",* is a bonafide research work done by **Nurul Mohammad Shayed**, ID: 2011-1-70-003 in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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Abdllah-Al-Faysal
Lecturer
Department of Pharmacy
East West University
Dhaka, Bangladesh.

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled **"Cytotoxic, antimicrobial and antioxidant activity of crude fraction of *Ficus recmosa* leaves extract"** is a bona fide research work done by Nurul Mohammad Shayed (ID: 2011-1-70-003), in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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Chairperson

Department of Pharmacy

East West University

Aftabnagar, Dhaka.

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Dedicated

To

My Loving Family

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ABSTRACT

The purpose of the study was to evaluate the cytotoxic, antimicrobial and antioxidant activity of crude fraction of *Ficus recemosa* (Family: Moraceae) leaves extract. The powdered leaves of *Ficus recemosa* were extracted with methanol and then partitioned with pet-ether, Dichloro methane , ethyl acetate and crude fraction was taken for experiment. The crude fraction was used to evaluate cytotoxic, antimicrobial and antioxidant activities. The cytoxic activity was measured by brine shrimp lethality bioassay. Crude fraction showed cytotoxic activity with LC_{50} value 58.87µg/ml in brine shrimp lethality test The antimicrobial activity was assessed by disc diffusion method.. In antimicrobial activity investigation the crude 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 27.32 mg AAE/g of total phenolic content and 477.65 mg AAE/g total flavonoid content. The results of study clearly indicate the presence of cytotoxic, antimicrobial and antioxidant properties of crude extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

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

Chapter: 1

INTRODUCTION

1.1 INTRODUCTION

1.1.1 Natural Products in Medicine

Death is authentic but unavoidable. Nobody can desire to lose his short but sweet life. Man is therefore, being continued his struggle to achieve mastery over the forces of nature- Diseases Decay and Death. Human struggle against the misery of three D's-Disease, Decay and Death is eternal. From the very inception of civilization, the inherent concern for getting as well as staying healthy has been instigating human venture for cure from his surroundings. Illness, physical discomforts, injuries, wounds & fear of death had forced prehistoric man to use any natural substances that he/she could lay his/her hands on- "the green friends" PLANTs (Ogdan, 1981).

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.

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 (WHO, 2001). Examples of important drugs obtained from plants are digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristrine and vinblastine from *Catharanthus 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 (Farnsworth *et al.*, 1967).

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).

1.1.2 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.

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).

1.1.3 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, that 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 synthesis of useful drug". World Health Organization (WHO) reported that 80% of the world‟ s population depends on medicinal plants for their primary health care. 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).

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs. In modern through the use of European Scientific methods . The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins. 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 (Abayomi, 1993).

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow et al., 2003). Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity .There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry (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 Pun-tsao contains thousands of herbal cures attributed to Shennung, 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. (Levetin and Mahon M, 2003). 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 MateriaMedica. 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 ofEurope for the next 1500 years (Bryan *et al*,. 1989).

The beginning of the Renaissance saw a revival of herbalism in the identification of medicinallyuseful plants. This coupled with the invention of the printing press in 1450 ushered in the Age ofHerbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses ofplants, but also included much misinformation and superstition. The Doctrine of Signatures, forexample, held that the medicinal use of plants could be ascertained by recognizing features of theplant that corresponded to human anatomy. For example, the red juice of bloodwort suggests thatit 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 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 Herbal medicine

Herbal medicine -- also called botanical medicine or phytomedicine -- refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes. In the early 19th century, when chemical analysis first became available, scientists began to extract

and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use (University of Maryland Medical Center, 2014).

1.1.6 Global status of herbal medicine

Medicinal plants have played a key role in world health. It is estimated that about 25 – 30% of all modern medicines are directly or indirectly derived from higher plants. The herbal products industry comprises a number of inter related sub sectors including as Herbal tears; Functional foods; Nutraceuticals; Phytochemicals; Ethical OTC medicines; Flavors and fragrances; Aroma therapy; Culinary herbs and Spices. As per World Bank reports trade in medicinal plants, botanical drug products and raw material is growing at an annual growth rate between 5 to 15%. The Global pharmaceutical market has risen from US $550 billion in 2004 worth to a close to US $900 billion in the year 2008. The herbal industry shares about US $62 billion with good growth potential. In India the value of botanicals related trade is about US $10 billion per annum with annual export of US $1.1 billion while China annual herbal drugs production is worth US $48 billion with export of US $3.6 billion. Presently the United States is the largest market for Indianbotanical products accounting for about 50% of the total exports. Japan, Hong Kong, Korea and Singapore are the major importer of the herbal drugs making 66% share of China botanical drug export. Within the European community botanical medicine represents an important share of the pharmaceutical market (Kumari, Shukla and Rao, 2011).

1.1.7 Significances of medicinal plants to human being

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.
- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants. (Chaudhary SA et al., 2010).

1.1.8 The Medicinal Plants contribution in the New World

Just Before Modern Medicine: At the early of modern medicine the Muslim physicians were done a great job. The Arabian Muslim physicians, like Al-Razi and IbnSina (9th to 12th century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi‟ s important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah, Maan La YahoduruhoTibb etc. The famous medical book, Al-Kanun, of IbnSina was the prescribed book of medicine in the schools of western medicine for several centuries (Mian&Ghani., 1990).

The use of medicinal plants in Europe in the 13th and 14th centuries was based on the Doctrine of Signatures or Similar developed by Paracelsus (1490-1541 AD), aswiss alchemist and physician (Murray, 1995). The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca (Erythroxylum species) and tobacco (Nicotianatabacum) was common in these countries in the 14th and 15th centuries. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the Rig Veda (4500-1600 BC). It supplies various information of the medicinal use of plants in the

Indian subcontinent (Hill, 1972). Medicinal plants used by the Australian aborigines many centuries ago tremendously enriched the stock of medicinal plants of the world. The current list of the medicinal plants growing around the world includes more than a thousand items (Sofowora, A., 1982)

1.1.9 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).

1.1.10 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 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):

1. Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.

2. Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.

3. 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.*

1.2.3 Necessity Of Drug Development From Plant Sources

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role. Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents. So the application of herbal drug in crude form may be ineffective or may cause a toxic reaction. Vincristine, a prominent anticancer drug, was developed from periwinkle plant (*Vinca rosea*) which was formerly prescribed for treating diabetes. The efficient hypotensive drug, reserpine, was developed from *Rauwolfia serpentine* which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN *et al*., 1982). Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammi visnaga* which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result.

1.2.4 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:

1. Selection and correct identification of the proper medicinal plant.
2. Extraction with suitable solvent(s).
3. 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.
4. Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.
5. Repeated fractionation of active fractions to isolate pure compound(s).
6. Elucidation of chemical structure of pure compound(s) using spectroscopic methods.

7. Evaluation of biological activity of pure compound(s)
8. Toxicological tests with pure compound(s).
9. Production of drug in appropriate dosage forms.

1.2.5 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.

1.3 Plant review of *Ficus recemosa*

1.3.1Plant Family: Moraceae

Ficus recemosa. Is a traditional plant from the plant family of Moracese. The Moraceae often called the mulberry family or fig family are a family of flowering plants comprising about 40

genera and over 1000 species. Most are widespread in tropical and subtropical regions, less so in temperate climates. The only synapomorphy with in Moraceae is presence of laticifers and milky sap in all parenchymatous tissues, but generally useful field characters include twocarpels sometimes with one reduced, compound inconspicuous flowers, and compound fruits. Included are well-known plants such asthe fig, banyan, breadfruit, mulberry, and Osage-orange. The 'flowers' of Moraceae are often pseudanthia (reducedinflorescences). (Zhou,Michael 2003).

Table 1.1: Scientific classification of *Ficus recemosa*

Scientific classification	
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Moraceae
Genus:	*Ficus*
Species:	*F. racemosa*

1.3.2 Botanical Names-Synonym

Different scientists in different part of the world may think they discovered the plant first, so each of them gave different names. But the first given name take precedence, the other names are rejected, and thereafter referred to as synonyms. (India Biodiversity portal 2012).

Table 1.2: Synonyms of *Ficus recemosa*.

Synonym	*Ficus glomerata*
Synonym	*Ficus lucescens*
Synonym	*Ficus racemosa var. elongata*
Synonym	*Ficus glomerata Roxb.*
Synonym	*Ficus racemosa*
Synonym	*Covellia glomerata (Roxb.) Miq.*
Synonym	*Ficus goolereea Roxb.*

1.3.3 Plant Description of *Ficus recemosa*

A medium-sized to large deciduous, sometimes evergreen tree with spreading crown and white latex. Leaves 7.5-15 cm long, ovate-oblong or elliptic-lanceolate, entire, tapering to a bluntish point at the apex. Receptacles shortly pedunculate, on short leafless warted branches which issue from the stem and larger branches, subglobose, pyriform or subturbinate, 3.2 cm across, red when ripe.

The fruits are considered astringent, stomachic and carminative; given in menorrhagia, haemoptysis, bronchitis, dry cough, diseases of kidney and spleen. The unripe fruit is astringent to the bowels, tonic and styptic; allays thirst, useful in leucorrhoea. The ripe fruit is acrid and cooling; useful in biliousness, burning sensation, fatigue, urinary discharges, thirst, leprosy, menorrhagia and nose bleeding. The fresh juice of the ripe fruit is used as an adjunct to a metallic preparation, which is given in diabetes

Figure 1.1: *Ficus recemosa* Tree, Leaves, fruit.

Fruits are used for rheumatic pain in Khagrachari by the Chakma. Bark is cooling, astringent and galactagogue; useful in asthma, piles and gravid uterus; as an infusion it is given for menorrhagia. The leaves are astringent to the bowels and good for bronchitis and bilious affections. Latex is aphrodisiac and vulnerary, useful in inflammations, piles, diarrhoea and in combination with sesamum oil in cancer. Roots are used in dysentery; sap is tonic and used in diabetes (Suresh Jawakhar& Sabir, 1992).

Figure 1.2: *Ficus recemosa* ripe fruit.

EtOH(50%) extract of stem bark is antiprotozoal and hypoglycaemic. A glycoside-rich fraction from leaves have hypotensive and cardiac-depressive effects. Petroleum ether and alcoholic extracts of the leaves possess anti-inflammatory activity

1.3.4 Chemical composition of *Ficus recemosa*

Leaves contain glycosides, gluanol acetate, β-amyrin and β-sitosterol. Bark contains ceryl behanate, lupeol, lupeol acetate, α & β- amyrin, gluanol acetate, β-sitosterol, stigmasterol and a ketone. Gluanol acetate and β-sitosterol have also been isolatated from the heartwood. An alkaloid, dumurin has been isolated from the stem bark. Fruits contain lupeol acetate, β-sitosterol, hentriacontane, gluanol acetate and tiglic acid ester of taraxasterol and glucose (Ghani, 2003).

A new tetracyclic triterpene-glauanol acetate have been isolated from leaves, bark and heartwood. Spectrometry data of few active constituents of *F. racemosa*

1.3.5 Traditional 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. From ancient times all the parts of this plant have been used for their medicinal value. It is basically used for its antidiuretic effect. In the Ayurvedic System of Medicine, the roots are popularly used for the treatment of hydrophobia, whereas, the bark has multiple actions. It is used as a galactagogue and is helpful in gynecological disorders.The fruits are active against leprosy, menorrhagia, leucorrhoea, and blood disorders, burns, intestinal worms, dry cough, and urinary tract infections. Bronchitis, bowel syndrome, and piles are treated with its leaves, in the Unani System of Medicine. The leaf buds are effective against skin infection, and a decoction of the leaves is used in wound washing and healing (Padmaa, 2009)

1.3.5.1 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 odour It is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery and piles.

1.3.5.2 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.

1.3.5.3 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, dullreddish or dark crimson on ripening. The fruit of F. Racemosa 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.

1.3.5.4 Latex

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

1.3.5.5 Roots

The roots of *Ficus racemosa* Linn are long and brownish in color. 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. The roots of the plant are used in dysentery, pectoral complications, and diabetes, and also applied in inflammatory glandular enlargement, mumps, and hydrophobia.The summary of traditional uses is tabulated in following table (Udumbar, 2014).

Table 1.3: Traditional use of different parts of *F. racemosa*

Plant material	Uses in Ayurvedic medicines
Fruits	Aphthae, menorrhagia, hemoptysis
Fruits, boiled and strained	Gargle for sore throat
Ground leaves mixed with honey	Bilious affections
Latex (milky juice)	Diarrhea, hemorrhoids
Bark powder	Diabetes
Roots	Dysentery
Latex boiled with milk	Aphrodisiac
Oil infused with root bark	Eczema, leprosy, rheumatism
Fruits	Laxative, digestive

1.3.6 Distribution

Throughout Bangladesh, near streams and canals. Found throughout India, this is native to Australia, Malaysia and Indonesia (Padmaa, 2009).

Chapter: 2

LITERATURE REVIEW

2.1 Phytochemical Properties

The stem bark of Ficus Racemosa Linn contains tannin, wax, saponin gluanol acetate, β sitosterol, leucocyanidin- 3 – O – β – D - glucopyrancoside, leucopelargonidin – 3 – O – β – D -glucopyranoside, leucopelargonidin – 3 – O – α – L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin from trunk bark,lupeol, β-sitosterol and stigmasterol were isolated. Fruit contains glauanol, hentriacontane,β sitosterol, glauanolacetate, glucose, tiglic acid, esters of taraxasterol,lupeolacetate, friedelin, higherhydrocarbons and other phytosterol .A new tetra triterpene glauanol acetate which is characterized as 13α, 14β, 17βH, 20 α H-lanosta-8, 22-diene-3βacetate and racemosic acid were isolated from the leaves. An unusual thermo stable aspartic protease was isolated from latex of the plant. The stem bark and fruit showed the presence of glauanol acetate(Sumit *et al*. 2012).

2.2 Pharmacological Activities

The whole parts of the plant exhibit wide spectrum of pharmacological activities and used such as hypoglycemic, hypolipidemic, renal anti-carcinogenic, anti-diuretic, anti-tussive, hepatoprotective, radioprotective, anti-ulcer, antiinflammatory, antidiarrhoeal and antifungal etc.

Pharmacological actions of *F. racemosa*

2.2.1 Hypoglycemic

There are more than one type of hypoglycemic principles, both organic and inorganic, in Ficus Racemosa Linn fruits which produce a significant fall in blood glucose levels in normal and alloxan-diabetic rabbits by producing an organotropic effect on the B-cells which results in an increased release of insulin from the pancreatic beta cells. Ficus Racemosa Linn fruits pulp may, in the long run after more detailed studies, prove to be a more valuable anti-diabetic agent as in addition to its insulin releasing and insulin-like activities. Methanol extract of powered fruits at the dose 1, 2, 3, and 4 g/kg reduced the blood glucose level in normal and

alloxan induced diabetic rabbits 25. On the other hand the glucose-lowering efficacy of methanol extract of the stem bark was evaluated both in normal and alloxan-induced diabetic rats at the doses of 200 and 400 mg/kg p.o. The activity was also comparable to that of the effect produced by a standard antidiabetic agent, glibenclamide (10 mg/kg) proving its folklore claim as anti-diabetic agent.

The ethanol extract (250 mg/kg/day), lowered blood glucose level within 2 weeks in the alloxan diabetic albino rats confirming its hypoglycemic activity (Sumit *et al.* 2012).

Pharmacological actions	Parts used	Extract	Experimental models
Antihyperglycemic	Stem bark	Ethanol	Alloxan-induced
	Stem bark	Methanol	Alloxan-induced
	Fruits	Methanol	Alloxan-induced
Antitussive	Stem bark	Methanol	Cough-induced model by sulfur dioxide gas
Hepatoprotective	Stem bark	Methanol	Hepatotoxicity induced by CCl_4
	Leaf	Ethanol	Hepatotoxicity induced by CCl_4
Antioxidants	Fruits	Ethanol	DPPH free radical scavenging assay
Wound healing	Stem bark	Ethanol	Excised and incised wound model
Antidiarrheal	Stem bark	Ethanol	Castor oil–induced diarrhea
			PGE2-induced enter pooling
Anti-inflammatory	Leaf		Carrageenan-, serotonin-, histamine-, and dextran-induced rat paw edema
Antiulcer	Fruit	Ethanol	Pylorus ligation, ethanol induced, cold restraint stress
Antibacterial	Leaf	Petroleum ether	*E. coli, B. pumilus, B. subtilis, P. aeruginosa, S. aureus*
Hypolipidemic	Bark	Ethanol	Alloxan-induced
Renal anticarcinogenic			Potassium bromate-induced nephrotoxicity, Ferric nitrilotriacetate-induced nephtotoxicity

Table 2.1: Pharmacological actions of Ficus Recemosa plant parts.

β-sitosterol isolated from the stem bark was found to possess potent hypoglycemic activity when compared to other isolated compounds 29. Ethanolic extract of leaves lowered the blood glucose levels by 18.4 and 17.0% at 5 and 24 hr, respectively, in sucrose challenged streptozotocin induced diabetic rat model at the dose of 100 mg/kg body weight.

2.2.2 Hypolipidemic:

Dietary fiber content of fruits when fed to rats in diet induced pronounced hypocholesterolemic effect, as it increased fecal excretion of cholesterol as well as bile acids Hypolipidemic activities of ethanolic extract of bark were studied at the doses of 100-500 mg/kg b.w to alloxan-induced diabetic rats. Investigation showed that extract had potent anti-diabetic and hypolipidemic effects when compared to that of the standard reference drug, glibenclamide (Deva Gowda & Prakash ,2008)

2.2.3 Anti-oxidant and a probable radio-protector

Ethanol extract and water extract were subjected to free radical scavenging both by steady state and time resolved methods. The ethanol extract exhibited significantly higher steady state antioxidant activity. It also exhibited concentration dependent DPPH, ABTS, hydroxyl radical and superoxide radical scavenging and inhibition of lipid peroxidation when tested with standard compounds. In vitro radio protective potential of Ficus Racemosa Extract (FRE) was studie dusing micronucleus assay in irradiated Chinese hamster lung fibroblast cells (V79). Pretreatment with different doses of FRE 1hr prior to 2 Gy γ-radiation resulted in a significant decrease in the percentage of micro nucleated binuclear V79 cells suggesting its role as radio protector.

The methanol extract of stem bark has shown potent in vitro antioxidant activity when compared to the methanol extract of its roots.

The fruit ethanol extract exhibited significant antioxidant activity in DPPH free radical scavenging assay. 3-O-(E)-Caffeoyl quinate showed significant antioxidant activity (Sumit *et al.* 2012).

2.2.4 Anti-diuretic

The decoction of the bark of Ficus Racemosa Linn is claimed as an anti-diuretic and its potential is evaluated in rats using three doses (250, 500 or 1000 mg/kg). It had a rapid onset

(within 1 hr), peaked at 3 hr and lasted throughout the study period (5 hr). It also caused a reduction in urinary Na+ level and Na+/K+ ratio, and an increase in urinary osmolarity indicating multiple mechanisms of action (Padmaa, 2009).

2.2.5 Renal anti carcinogenic

Ficus Racemosa Linn extract at a dose of 200 and 400 mg/kg when given orally a significant decrease in lipid peroxidation, xanthine oxidase, γ-glutamyl transpeptidase and hydrogen peroxide (H2O2) generation with reduction in renal glutathione content and antioxidant enzymes generated by KBrO3, a potent nephrotoxic agent that induces renal carcinogenesis in rats. There was significant recovery of renal glutathione content and antioxidant enzymes. There was also reversal in the enhancement of renal ornithine decarboxylase activ ity, DNA synthesis, and blood urea nitrogen and serum creatinine This result suggests that Ficus Racemosa Linn extract is a potent chemo-preventive agent and suppresses KBrO3-mediated nephrotoxicity in rats (Padmaa, 2009)

2.2.6 Antifungal

The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of Ficus Racemosa Linn was found to have antifungal activity. The extract inhibited the growth of several plant pathogens (Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp, Corynespora cassiicola and Fusarium sp). Psoralen was identified as the active compound and was shown to be biodegradable, having the potential to be developed as a fungicide against pathogens causing diseases on crops of economic importance (Deva Gowda & Prakash ,2008)

2.2.7 Hepatoprotective

Methanol extract of Ficus Racemosa Linn stem bark were studied using the model of hepatotoxicity induced by CCl_4 in rats. CCl_4 administration induced a significant increase in total bilirubin associated with a marked elevation in the activities of aspartat e aminotransferase(AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as compared to control rats. Pretreatment with methanol extract resulted in significant decreases in the activities of AST, ALT and ALP, compared to CCl_4 -treated rats. The results indicate that Ficus Racemosa Linn possesses potent hepatoprotective effects against CCl_4 -induced hepatic damage in rats (Padmaa, 2009)

2.2.8 Anti-tussive

The methanol extract of stem bark was tested for its Anti-tussive potential against a cough induced model by sulphur dioxide gas in mice. The extract exhibited maximum inhibition of 56.9% at a dose of 200 mg/kg (p.o.) 90 min after administration (Deva Gowda & Prakash ,2008)

2.2.9 Antibacterial

The hydro alcoholic extract of leaves was found effective against Actinomyces vicosus. The minimum inhibitory concentration was found to be 0.08mg/ml (Deva Gowda & Prakash ,2008)

2.2.10 Anti-diarrhoeal

Ethanol extract of stem bark has shown significant inhibitory activity against castor oil induced diarrhea and PEG2 induced enter pooling in rats and also showed a significant reduction in

gastro intestinal motility in charcoal meal test in rats which proves its efficacy as anti-diarrheal agent (Sumit *et al*. 2012).

2.2.11 Anthelmintic

The bark extract were evaluated for anthelmintic activity using adult earthworms, which exhibited a spontaneous motility (paralysis) With 50 mg/ml of aqueous extract the effects were compared with 3% piperazine citrate. There was no final recovery in the case of worms treated with aqueous extract in contrast to piperazine citrate, the worms recovered completely within 5 hr. This result shows the anthelmintic nature of the extract (Sumit et al. 2012).

2.2.12 Anti-pyretic

Methanol extract of stem bark was evaluated on normal body temperature and yeast-induced pyrexia in albino rats, at doses of 100, 200 and 300 mg/kg b.w p.o. It showed significant dose dependent reduction in normal body temperature and yeast -provoked elevated temperature which extended up to 5 hr after drug administration. The anti-pyretic effect was comparable to that of paracetamol (Sumit *et al.* 2012).

2.2.13 Anti-filarial

Alcoholic as well as aqueous extracts caused inhibition of spontaneous motility of whole wormand nerve muscle preparation of Setaria cervi characterized by increase in amplitude and tone ofcontractions. Both extracts caused death of microfilaria in vitro. LC50 and LC 90 were 21 and 35 ng/ml respectively for alcoholic, which were 27 and 42 ng/ml for aqueous extracts (Sumit *et al.* 2012).

2.2.14 Anti-analgesic

The ethanol extract of bark and leaves evaluated for analgesic activity by analgesio-meter at 100, 300 and 500mg/kg was found to posse's dose dependent analgesic activity (Sumit *et al.* 2012).

2.2.15 Anti-inflammatory

The anti-inflammatory activity of *Ficus racemosa* Linn extract was evaluated on carrageenin, serotonin, histamine and dextran-induced rat hind paw edema models. The extract (400 mg/kg) exhibited maximum anti-inflammatory effect of 30.4, 32.2, 33.9 and 32.0% 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, which was comparable to that of phenylbutazone.

Bioassay-guided fractionation of the ethanol extract of leaves isolated racemosic acid. It showed potent inhibitory activity against COX-1 and 5-LOX in vitro with LC_{50} values of 90 and 18 μm, respectively Ethanol extract of stem bark also inhibited COX-1 with LC_{50} value of 100 ng/ml proves that the drug is used in the treatment of inflammatory conditions (Sumit *et al.* 2012).

2.2.16 Wound healing

Ethanol extracts of stem bark show a potent wound healing in excised and incised wound model in rat (Padmaa, 2009)

2.2.17 Larvicidal

The larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone and methanol extracts of the leaf and bark were assayed for their toxicity against the early fourth-instar larvae of Culex quinquefasciatus (Diptera: Culicidae). The larval mortality was

observed after 24 hr exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in acetone extract of bark. The bioassay-guided fractionation of acetone extract led to the separation and identification of a tetracyclic triterpenes derivative. Gluanol acetate was isolated and identified as new mosquito larvicidal compound. Gluanol acetate was quite potent against fourth-instar larvae of Aedes aegypti L. LC_{50} 14.55 and LC_{90} 64.99 ppm, Anopheles stephensi Liston LC_{50} 28.50 and LC (90) 106.50 ppm) and C. quinquefasciatus Say LC_{50} 41.42 and LC_{90} 192.77 ppm (Padmaa 2009).

2.2.18 Anti-ulcer/Gastro-protective

Gastro-protective effect of 50% ethanolic extract of Ficus Racemosa Linn known as F. glomerata fruit (FGE) was studied in different gastric ulcer models in rats. FGE prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by significant decrease in superoxide dismutase, H+K+ATPase and increase in catalase activity. The H+K+ATPase are thedimeric enzyme responsible for H+secretion by the gastric parietal cells. H+K+ATPase are selectively blocked by the action of ranitidine, an acid blocker used to treat gastric ulcers

The present study shows the pharmacological properties and therapeutic potential of various bioactive compounds present in the plant. On the bases of its pharmacological properties it may be concluded that the present single herbal intervention proven itse lf a magical remedy for various disorders and it's worthwhile to mention that the Ficus Racemosa Linn on the basis of its antioxidant and hypolipidemic activities may be explored for the various chronic vascular complications. However, further clinical studies are needed to evaluate the therapeutic potential of this plant in clinical practice (Sumit et al. 2012).

2.3.1 Toxicity study

Acute toxicity studies were performed previously using the aqueous extract of *F. racemosa* bark on albino mice. The aqueous extract was given in doses of 100, 300, and 1000 mg per 100 gm body weight, once, to the mice and the animals were sacrificed after 72 hours of dosing. Blood

samples were collected to determine the hemoglobin, red blood cell (RBC) count, white blood cell (WBC) count, blood urea, blood glucose, serum creatinine, serum cholesterol, and serum glutamate pyruvate transaminase (SGPT). Some physiological changes were also observed in the liver and kidney by the researcher. The extract was safe up to the highest dose, but it produced abnormality in the liver and kidney. The fasting blood sugar level pointed toward hypoglycemia. Small amounts of fatty acids were also deposited in the kidney.

The researcher also performed acute and subacute toxicity studies of the aqueous extract of *F. racemosa* bark in both albino mice and albino Wistar rats. They administered the extract in 30 mg/100 gm dose for three weeks. Liver damage was seen in the subacute toxicity study and reversible hepatotoxicity was also observed during the experiment. There were no signs of renal damage during the histopathological studies, but the creatinine and urea levels had increased dramatically (Phytochemistry, 2009).

2.3.2 Clinical trials

The platelet-aggregating activity of the *F. racemosa* aqueous extract was studied on healthy human volunteers recently. Both extracts exhibited a platelet-aggregating activity to an extent of 3 – 51% when compared to the controls. The platelet-aggregating activity of both extracts was dose-dependent and had no significant difference. The cold water extract of the bark was reported to have bergenin as a major chemical constituent, while the hot water extract contained ferulic acid, kaempferol, and coumarin, along with bergenin

The clinical trials of β-sitosterol in a randomized, double-blind, and placebo-controlled multicenter study showed effectiveness of treatment against benign prostate hyperplasia. β sitosterol produced adverse effects like dizziness, decreasing blood pressure, tachycardia, and orthostatic problems during the study

Another double-blinded, randomized, placebo-controlled design clinical trial was performed, to check the antihyperglycemic activity of the *F. racemosa* bark on human volunteers (18 men and 12 women). The researchers maintained a few inclusion criteria like volunteers' age to be between 35 and 50 years; they should be free from diabetic retinopathy, nephropathy or

cardiomyopathy; should not be pregnant; should have a normal lipid profile; had to be metabolically stable; and should not have been taking insulin, lipid-lowering drugs or herbs / supplements. They established a human equivalent dose (HED) of 1.2 g / day (400 mg, three capsules) based on *in vivo* animal studies. After one month of study, the biochemical parameters like fasting blood glucose, postprandial blood glucose, and insulin were measured, and they reported that the body mass index (BMI) of the subjects from both groups were above normal levels and no significant changes were observed during the study period. The subjects showed good tolerance to the treatment and no volunteer had withdrawn from the study. A significant reduction of blood glucose level was observed during the extract treatment without alteration of cholesterol or triglyceride levels(Sumit *et al*. 2012).

2.3.3 Mechanism of action

After the preclinical and clinical studies, some mechanism of action had been proposed through the antihyperglycemic activity of the *F. racemosa* extract (Sumit *et al.* 2012).

2.4.3.1 On postprandial hyperglycemia

The extract reduced postprandial hyperglycemia via increasing the viscosity of the intestinal contents, resulting in entrapment of the glucose molecules by the adsorption method, thereby reducing diffusion of glucose from the intestinal barrier to the blood stream.

2.4.3.2 On glucose absorption

The extract reduced glucose absorption via inhibiting carbohydrate hydrolyzing enzymes (α-amylase, α-glucosidase, β-glucosidase) and delaying the release of glucose into the blood stream.

2.4.3.3 Utilization of glucose

The *F. racemosa* extract controlled the plasma glucose level by regulating the glucose metabolizing enzymes in the glycolysis and gluconeogenesis pathways.

2.4.3.4 Peripheral utilization of glucose

This action was increased via the glucose uptake across target cells and secretion of insulin into the blood stream.

2.4.3.5 Pancreatic β-cell regeneration

It increased through synthesis and secretion of insulin in to blood stream.

Chapter: 3

METHODS & MATERIALS

3.1. Collection & Preparation of Plant Material

Plant sample (Leaves) of *Ficus recemosa* was collected from Gopalgong, in January 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 pet-ether

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

3.4.2 Partition with Dichloro methane

To the mother solution left after partitioning with pet-ether, 12.5ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with Dichloro methane($CHCl_2$). The process was repeated thrice (100ml X 3). The CCl_4 fraction was then air dried for solid residue.

3.4.3 Partition with Ethyl acetate

To the mother solution that left after partitioning with n-hexane and Dichloro methane($CHCl_2$), 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 from crude

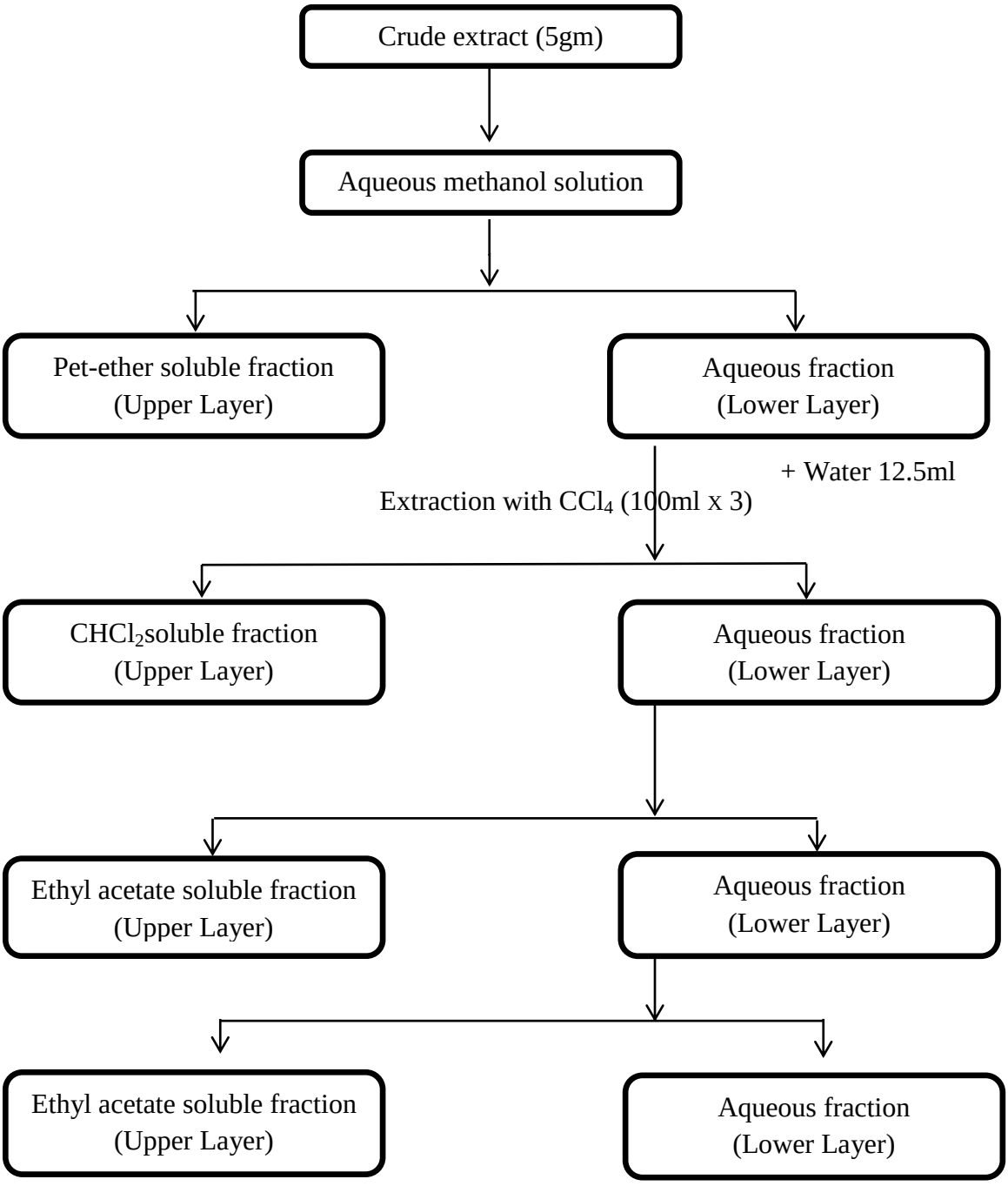


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Ficus Resemosa* leaves

To the mother solution that left after washing with n-Hexane, CCl_4 and $CHCl_3$, was then taken in a separating funnel and extracted with Ethyl acetate (100ml X 3). The crude fractions were collected together and air dried.

3.4.5 Collection of Crude Fraction

After partitioning the mother solution with the four different solvents the crude fraction was collected and air dried. This crude 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- *Artemiasalina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae (Olowa and Nuneza, 2013; Rishikesh *et al.*, 2013).

3.5.2 Apparatus & Reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

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

3.5.3 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 *Artemiasalina* hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the pH 8.4 as sea water.

3.5.3.2 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.

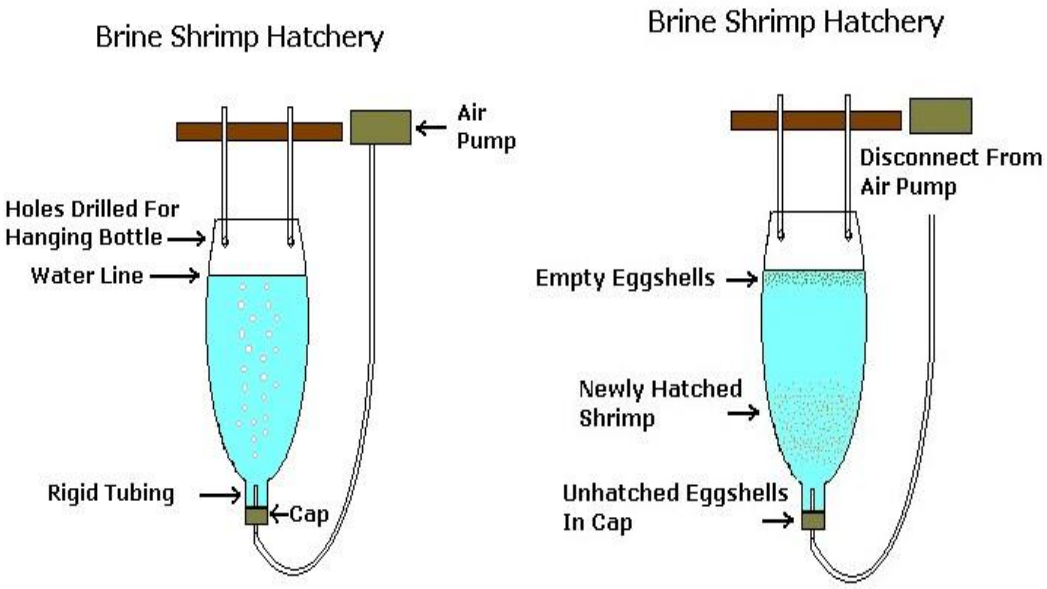


Figure 3.3: Brine shrimp Hatchery

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 activity by Disc Diffusion Method

3.6.1 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).

3.6.2 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

3.6.3 Test Sample of *Ficus resemosa*

Crude fraction of methanolic extract of *Ficus resemosa* leaves were taken as test sample.

3.6.4 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	*Bacillus subtilis* *Bacillus cereus* *Staphylococcus aureus* *sarcina lutea*
Gram –ve	*Salmonella paratyphi* *Shigella dysenteriae* *Vibrio parahaemolyticus* *Escherichia coli*
Fungi	*Candida albicans*

3.6.5 Procedure

3.6.5.1 Preparation of the medium

To prepare required volume of this medium, 5.6gm of agar medium was taken in a bottle with a cap and distilled water was added to it to make 200ml volume. The contents were then autoclaved to make a clear solution.

Figure 3.4: Autoclave machine

3.6.5.2 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Figure 3.5: Laminar hood

3.6.5.3 Preparation of the test plate

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

3.6.5.4 Preparation of Discs

Three types of discs were used for antimicrobial screening.

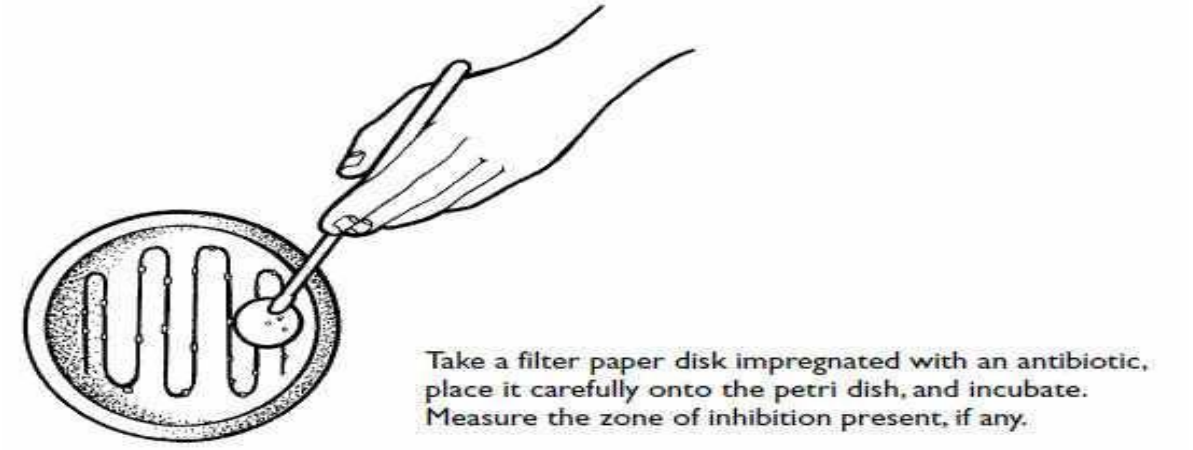


Figure 3.6: Preparation of filter paper discs

- **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.

3.6.5.5 Preparation of test sample

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

3.6.5.6 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.

3.6.5.7 Diffusion & Incubation

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

Figure 3.7: Incubator

3.6.5.8 Determination of Antimicrobial Activity By Measuring The Zone of Inhibition

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

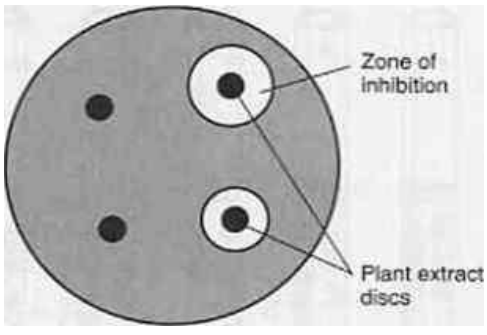


Figure 3.8: Clear zone of inhibition

Figure 3.9: Determination of clear zone of inhibition

3.7 Antioxidant Activity

3.7.1 Total Phenolic Content

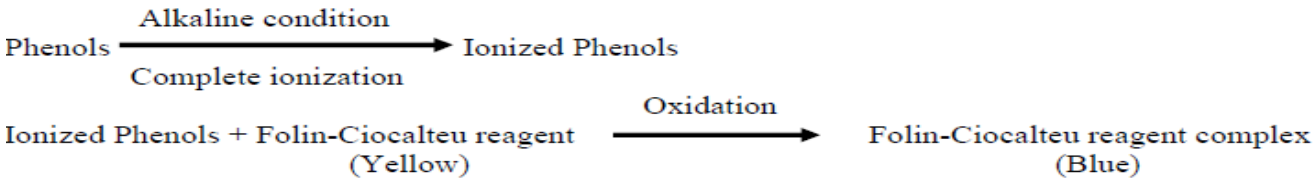
3.7.1.1 Principle

The content of total phenolic compounds in plant crude 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 Resemosa* crude 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)

3.7.2.2 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

3.7.2.3 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.

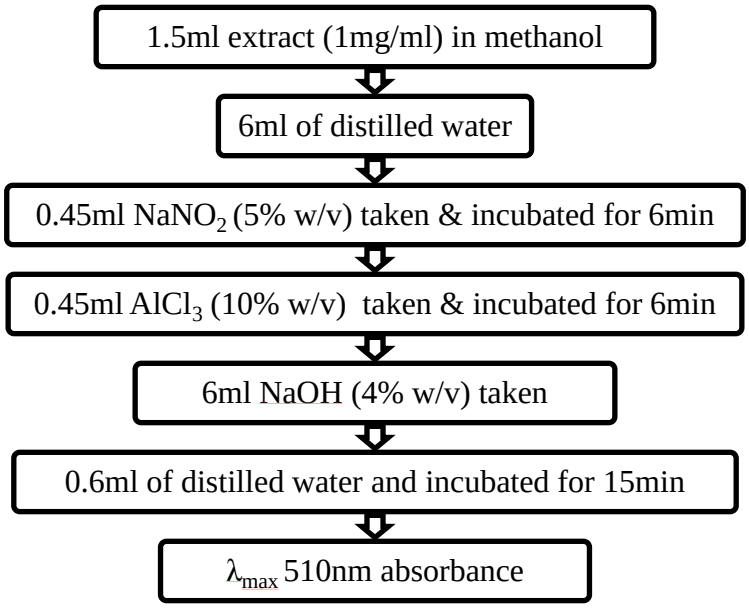


Figure 3.10: Schematic diagram of preparation of extract solution

Preparation of blank solution:

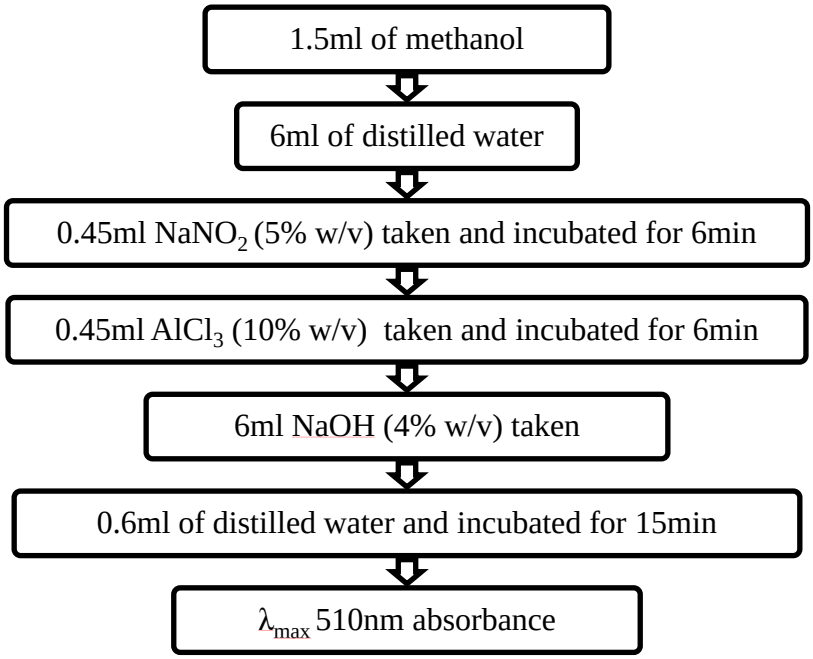


Figure 3.11: Schematic diagram of preparation of blank solution

4.1 Result of Brine Shrimp Lethality Bio-Assay

The crude fraction of the *Ficus recemosa* leaves extract were subjected to brine shrimp lethality bioassay following the procedure Meyer *et al.*, (1982). After 24hrs, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a median Lethal Concentration (LC_{50}) value. This represents the concentration of the standard or crude 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.

Chapter: 4

RESULTS & DISCUSSION

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 naupliidead**	**% 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	

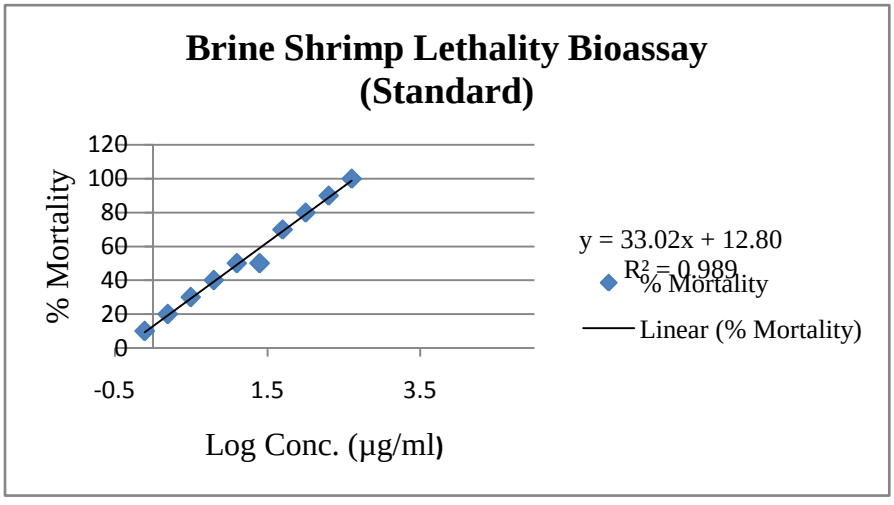


Figure 4.1: % mortality and predicted regression line of Tamoxifen (standard)

4.1.2 Preparation of crude fraction curve

Table 4.2: Results of the bioassay of crude 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	0	10	100	58.87
2	200	2.301	0	10	100	
3	100	2	0	10	90	
4	50	1.699	0	10	90	
5	25	1.398	0	10	80	
6	12.5	1.097	1	9	70	
7	6.25	0.796	2	8	70	
8	3.125	0.495	3	7	60	
9	1.5625	0.194	6	6	50	
10	0.78125	-0.107	6	6	40	

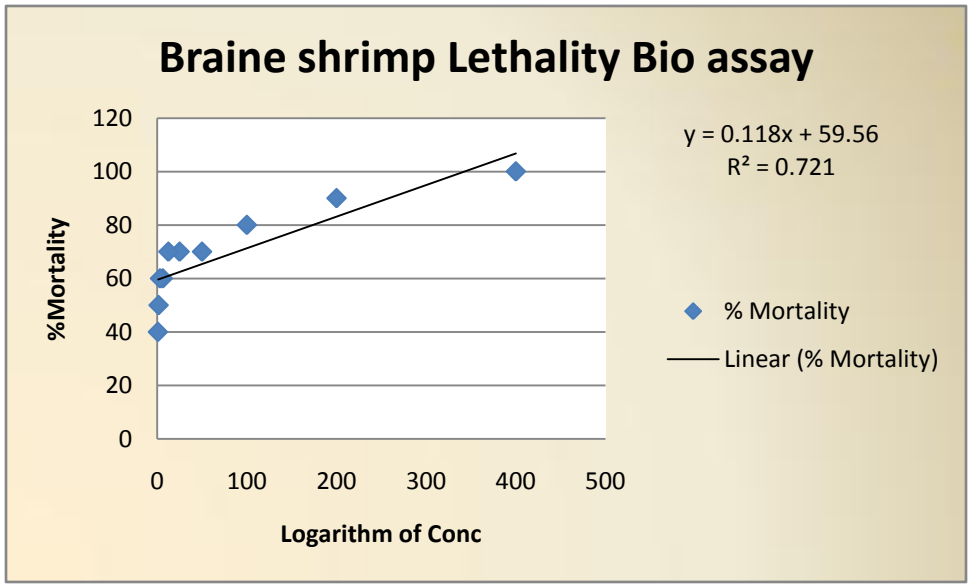


Figure 4.2: % mortality and predicted regression line of crude fraction (extract)

4.1.3 Discussion

Table 4.3: Cytotoxic activity of Tamoxifen and crude fraction of *Ficus Resemosa* leaves

Sample	**Linear regression equation**	**R^2 value**	**LC_{50} (µg/ml, 24hr)**
Standard (Tamoxifen)	y = 33.021x + 12.806	0.989	13.38
Crude fraction	y = 0.071x + 80.25	0.293	58.87

In this investigation, standard and crude fraction exhibited antioxidant properties with the LC_{50} values 58.87/ml and 13.38/ml respectively.

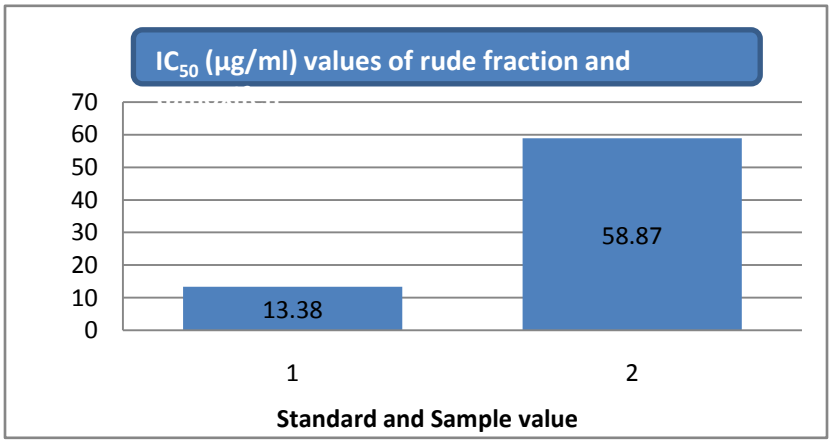


Figure 4.3: Comparison between LC_{50} values of standard and extract

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

4.2 Result of Antimicrobial Test

The antimicrobial activities of aqueous fraction of *Ficus recemosa* leaves extract were subjected 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. Ciprofloxacin was used as standard reference.

4.2.1 Zone Of Inhibition of Standard And Crude Fraction

Table 4.4: Antimicrobial activity of standard sample (ciprofloxacin) and Crude fraction

Type of microorganism		**Zone of inhibition (mm)**	
		Standard sample	**crude fraction**
Gram positive bacteria	*Bacillus subtilis*	20	8
	Bacillus cereus	18	7
	Staphylococcus aureus	20	5
	sarcina lutea	17	8
Gram negative bacteria	*Salmonella paratyphi*	18	6
	Shigella dysenteriae	16	5
	Vibrio parahaemolyticus	18	7
	Escherichia coli	14	4
Fungi	*Candida albicans*	16	7

4.2.2 Discussion

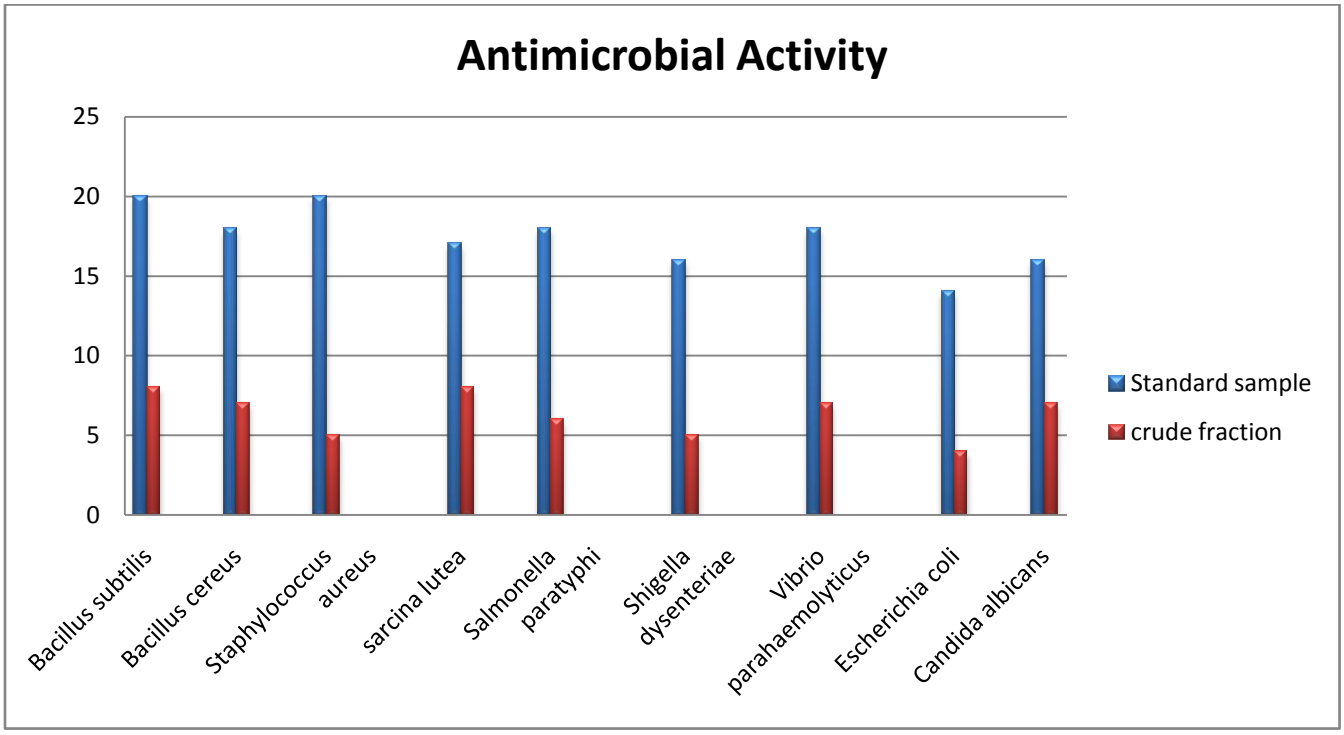


Figure 4.4: Comparison of antimicrobial activity between standard and extract

Crude fraction of *Ficus Resemosa* leaves extract showed low antimicrobial activity when compared to reference standard drug Ciprofloxacin. None of the zone of inhibition of crude fraction is equal to Ciprofloxacin against any bacteria or fungi as shown in the Figure: 4.4. Among all the microbiological cultures, the fraction showed the best antimicrobial activity against *Bacillus subtilis* (8 mm) & *Candida albicans* (7 mm) comparable to the standard (40mm).

4.3 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 crude fraction of *Ficus recemosa* leaves extract was determined by following methods-

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

4.3.1 Result of total Phenolic content

The crude extract of *Ficus Recemosa* leaves were subjected to determine total phenolic content. Ascorbic acid was used as reference standard.

4.3.1.1 Preparation of Standard Curve

Table 4.5: 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 4.5. This linear curve was considered as a standard curve.

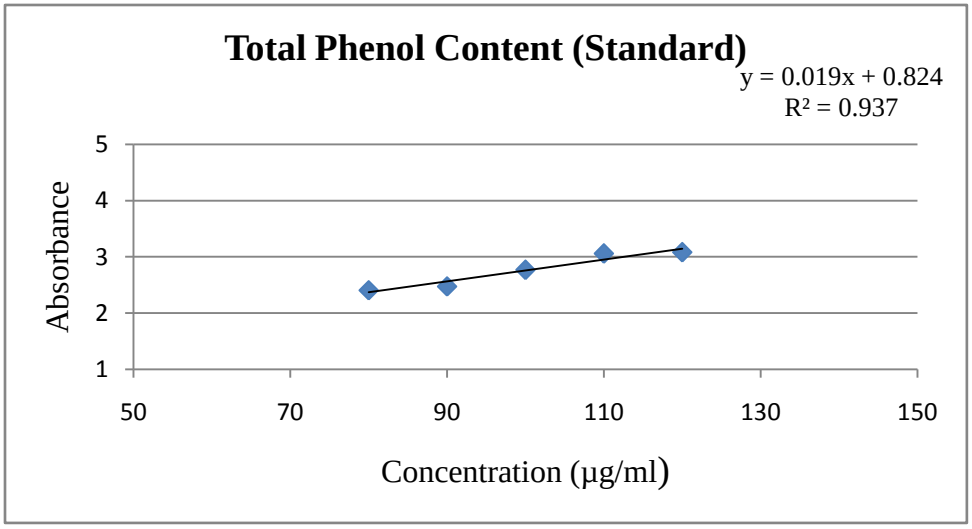


Figure 4.5: Graphical representation of assay of phenolic content of ascorbic acid

4.3.1.2 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 4.6: Total phenolic content of Crude fraction of leaves of *Ficus recemosa.*

Sample	**Concentration (mg/ml)**	**Absorbance**	**Average X value (mg of AAE/gm of dried extract)**
Crude fraction of *Ficus recemosa*	2	1.343	27.32

4.3.1.3 Discussion

The absorbance was found to be directly proportional to the concentration in both standard and crude fraction samples. In standard the absorbance increased with the increase in concentration indicating increase in phenolic content. Compare with the standard overall absorbance of the extract is less than the standard.

Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 27.32 mg of AAE/gm of dried extract of phenol content was found in the crude fraction of *Ficus recemosa* leaves.

4.3.2 Result of Total Flavonoid Content

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

4.3.2.1 Preparation of Standard Curve

Table 4.7: 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 absorbances were taken of different solution of ascorbic acid of concentrations ranging from 50µg/µl to 250µg/µl, a linear relationship was observed when the absorbances were plotted

against concentrations, as shown in Figure 4.10 This linear curve was considered as a standard curve.

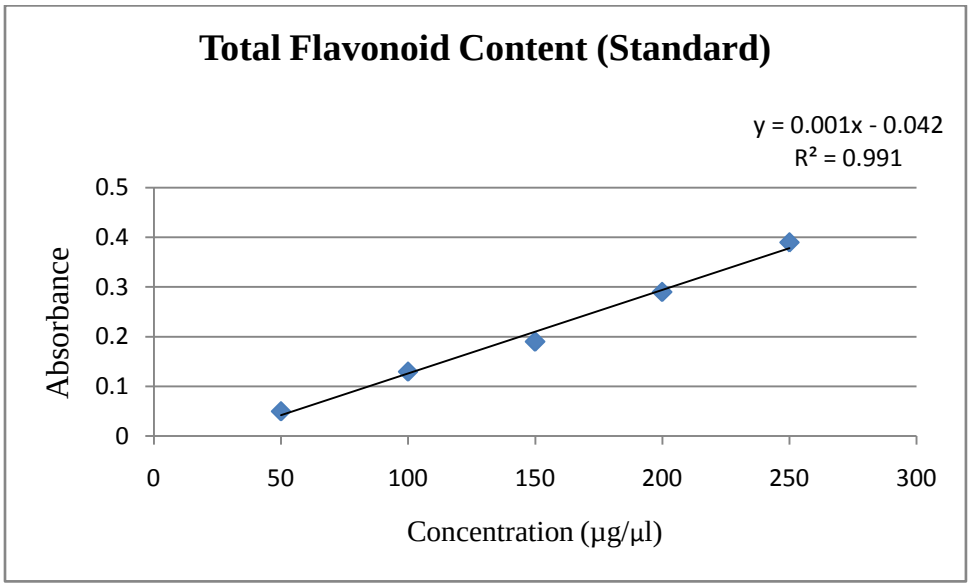


Figure 4.6: Graphical representation of assay of flavonoid content of ascorbic acid

4.3.2.2 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 4.8: Total flavonoid content of crude fraction of *Ficus recemosa* leaves extract

Sample	**Concentration (mg/ml)**	**Absorbance**	**Total flavonoid content (mg of AAE/g of dried extract)**
Crude fraction of *Ficus Recemosa*	1	0.77	477.65

4.3.2.3 Discussion

To determine the total flavonoid content of the test samples the standard curve was used. For 1mg/ml concentration of crude fraction of *Ficus recemosa* (leaves) 477.65 mg of AAE/gm of dried extract of flavonoid content was found. So it can be said that, the extract contains antioxidative compounds.

Chapter: 5

CONCLUSION

5.1 Conclusion

To conclude, the present study demonstrates that the crude extract of *Ficus resemosa* leaves can be considered as a valuable source of therapeutic agents for human health, as an cytotoxic agent. Since the *Ficus recemosa* leave extract exhibited potent cytotoxic activity, so the leaves can be further evaluated for anticancer, pesticidal and antitumor activity. In this study the extract showed low to moderate antibacterial activity but may be it will show effect at higher concentration. The crude extract also showed low antioxidant property. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases dysentery, menorrhagia, glandular swelling, abscess and chronic wounds. However further research and studies are required for identification and isolation of the active metabolite of the plant responsible for various activities. By the way, there are lots of plant like *Ficus resemosa* is available in different parts of our country. These plants are regularly used by the different tribal communities of Bangladesh in various kind of diseases. So, if perform study on these plants, maybe it will find out noble compounds, which is promising for us.

Chapter: 6
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