In Vitro Pharmacological Investigations of Petroleum Ether 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

Suvrota Voumik

ID: 2010-3-70-037

Department Of Pharmacy

East West University

DECLARATION BY THE CANDIDATE

I, Suvrota Voumik, hereby declare that this dissertation, entitled "***In Vitro* Pharmacological Investigations of Petroleum Ether 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.

Suvrota Voumik

ID: 2010-3-70-037

Department of Pharmacy

East West University

Aftabnagar, Dhaka

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"*In Vitro* Pharmacological Investigations of Petroleum Ether Fraction of *Ficus racemosa* Leaves Extract"** is a bonafide research work done, under myg uidance and supervision by Suvrota Voumik (ID: 2010-3-70-037), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Abdullah-Al-Faysal

Lecturer & Supervisor

Department of Pharmacy

East West University

Aftabnagar, Dhaka

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled **"*In Vitro* Pharmacological Investigations of Petroleum Ether Fraction of *Ficus racemosa* Leaves Extract"**is a bonafide research work done by Suvrota Voumik (ID: 2010-3-70-037), in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

Dr. Shamsun Nahar Khan

Associate Professor &Chairperson

Department of Pharmacy

East West University

Aftabnagar, Dhaka

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Dedication

This Research Paper is dedicated to

The Supreme Personality of

Godhead,

Who is the ultimate Master…

ABSTRACT

The purpose of the study was to evaluate the cytotoxic, antimicrobial and antioxidant activity of ethyl acetate fraction of *Ficus racemosa* (Family: Moraceae) leaves extract.The powdered leaves of *Ficus racemosa* were extracted with methanol and then partitioned with DCM, petroleum ether,ethyl acetate, and aquous consecutively. The petroleum ether fraction was used to evaluate cytotoxic, antimicrobial and antioxidant activities. The cytoxic activity was measured by brine shrimp lethality bioassay. The antimicrobial activity was assessed by disc diffusion method. Ethyl acetate fraction showed cytotoxic activity with LC_{50}value 23µg/ml in brine shrimp lethality test. In antimicrobial activity investigation the ethyl acetate fraction showed low to moderate antibacterial and antifungal activity against the tested organism compared to the azithromycin (22.9µg/disc) that was used as positive control. The fraction contained 40.01mg AAE/g of total phenolic content and 435mg AAE/g total flavonoid content. The results of study clearly indicate the presence of cytotoxic, antimicrobial and antioxidant properties of petroleum ether 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.

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LIST OF ABBREVIATIONS

µg	Microgram
µl	Microliter
AAE	Ascorbic Acid Equivalent

DMSO	dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
FCR	Folin-Ciocalteu Reagent
IC_{50}	The concentration of a drug which is required for 50% of inhibition of a specific test
LC_{50}	The lethal concentration required to kill 50% of the sample population of a specific test
WHO	World Health Organization

Chapter: 1

INTRODUCTION

1.1 Medicinal Plants: An Evergreen Part Of Medical Science

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).

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*,. 1967).

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 *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. 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 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).

1.1.6 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 crore 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):

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* (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 (*Vinca rosea*) which was formerly prescribed for treating diabetes. The efficient hypotensive drug, reserpine, was developed from *Rauwolfia serpentine* which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN *et al*., 1982). Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammi visnaga*which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Ghani, 1998).

1.3.2 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:

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 form(s).

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*Ficus racemosa*- A Brief Outlook

1.4.1Plant Family: Moraceae

Ficus racemosa is a traditional plant of Moraceae family. Moraceae, the mulberry family of the rose order (Rosales), with about 40 genera and some 1,000 species of deciduous or evergreen trees and shrubs, distributed mostly in tropical and subtropical regions. Plants of the family contain a milky latex and have alternate or opposite leaves and small, petalless male or female flowers. The fruits of many species are multiple because fruits from different flowers become joined together.The largest genus in the mulberry family, contains the banyan and the India rubber tree. The bark of the paper mulberry (Broussonetia) has been used for the manufacture of cloth and paper products. Among the ornamentals in the family are paper mulberry and Osage orange.

Table 1.1: List of all the genera of Moraceae family

Genera				
Antiaris	Antiaropsis	Artocarpus	Bagassa	Batocarpus
Bleekrodea	Bosqueiopsis	Brosimum	Broussonetia	Calaunia
Castilla	Clarisia	Dorstenia	Fatoua	Ficus
Helianthostylis	Helicostylis	Hullettia	Maclura	Maquira
Mesogyne	Milicia	Morus	Naucleopsis	Perebea
Poulsenia	Pseudolmedia	Scyphosyce	Sorocea	Streblus
Taxotrophis	Treculia	Trilepisium	Trophis	Vanieria

1.4.2 Plant Profile

1.4.2.1 Traditional Names

Classical or traditional names of plant include:

Table 1.2:Vernacular/ Local names of *Ficus racemosa*

Location	Names
Bangladesh	Udumbara
India	Gular (Hindi)
	Oriya (Dimri)
	Umbar(Marathi)
	Atthi (Malayalam)
	Athi (Dimri)
English	Cluster Fig
	Gular Fig
	Country Fig
Pakistan	Dumar

1.4.2.2 Taxonomical Ladder

Table 1.3: Scientific classification of *F.racemosa.*

Kingdom	Plantae
Division	Magnoliophyta
Class	Equisetopsida
Order	Rosales
Family	Moraceae
Genus	Ficus
Species	*Ficus racemosa*

1.4.2.3 Botany of *F. racemosa*

Deciduous trees, to 30 m high; bole buttressed; bark 8-10 mm thick, surface reddish-brown or yellowish-brown smooth, coarsely flaky, fibrous; blaze creamy pink; latex milky; young shoots and twigs finely white hairy, soon glabrous; branchlets 1.5-3 mm thick, puberulous. Leaves simple, alternate, stipules 12-18 mm long, lanceolate, linear-lanceolate, pubescent, often persistent on young shoots; petiole 10-50 mm long, slender, grooved above, becoming brown scurfy; lamina 6-15 x 3.5-6 cm, ovate, obovate, elliptic-oblong, elliptic-lanceolate, elliptic-ovate or oblong-ovate, base acute, obtuse or cuneate, apex narrowed, blunt or acute, margin entire, membranous, glabrous, blistered appearance on drying; 3-ribbed from base, 4-8 pairs, slender, pinnate, prominent beneath, intercostae reticulate, obscure. Flowers unisexual; inflorescence a syconia, on short leafless branches or warty tubercles of trunk or on larger branches, subglobose to pyriform, smooth, often lenticellate-verrucose; peduncle 3-12 mm long, stout, orifice plane or slightly sunken, closed by 5-6 apical bracts; internal bristles none; basal bracts 3, 1-2 m long, ovate-triangular, obtuse, persistent; flowers of unisexual, 4 kinds; male flowers near the mouth of receptacles, in 2-3 rings, sessile, much compressed; tepals 3-4, dentate-lacerate, lobes jointed below, red, glabrous; stamens 2, exserted; filaments 1 mm, connate below; anthers oblong, parallel; female flowers sessile or very shortly stalked among gall flowers; tepals 3-4, dentate-

lacerate, lobes jointed below, red, glabrous, ovary superior, sessile or substipitate, red spotted; style 2-3 mm long, glabrous, simple; stigma clavate; gall flowers long stalked; ovary dark red, rough; style short. Syconium 2.5 x 2 cm, orange, pink or dark crimson; achene granulate.

(a)

(b)

(c)

(d)

Fig 1.1:*Ficus racemosa*- (a) Whole plant; (b) Leaves; (c) Fruits; (d) Bark

1.4.2.4Pharmacognostical Characteristics

Macroscopical

The tree is medium to moderate sized deciduous. The rich green foliage provides a good shade.

a) **Leaves:**The leavs are dark green, 7.5-10 cm long, glabrous; receptacles small subglobose or piriform, in large clusters from old nodes of main trunk.
b) **Fruits:**The friuts 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 3/4inch to 2 inches long, circular and grows directly onthe trunk.
c) **Seeds:**The seeds are tiny, innumerable and grain-like. Outer surface of the bark consists of easily removable translucent flakes grayish to rusty brown, uniformly hard and non-brittle.
d) **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. Unlike the banyan, it has no aerial roots. Those looking for the flower of goolar should know that the fig is actually a compartment carrying hundreds of flowers.Texture is homogeneously leathery.
e) **Roots:**The roots of F.racemosa are long, brownish in colour. It's having characteristic odour and slightly bitter in taste.Roots are irregular in shape.

Microscopical

a) **Cork:** The cork is made up of polygonal or rectangular cells. The phellogen is made up of 1-2 layers of thin walled cells.
b) **Phelloderm:**Phelloderm is well marked compact tissue consisting mainly of parenchymatous cells with isolated or small groups of sclereids, particularly in inner region. Sclereids are lignified with simple pits. Several parenchymatous cells contain single prism of calcium oxalate or some brownish content.

c) **Cortex:**The cortex is wide with numerous sclereids and some cortical cells contain resinous mass. Prismatic crystals of calcium oxalate are present in some of the cells.Sclereids are rectangular or isodiametric and pitted thick walled.

d) **Phloem:**Phloem consists of sieve tubes, companion cells, phloem parenchyma,sclereids, phloem fibres and medullary rays. Starch grains are ovoid to spherical. Laticiferous vessels with a light brown granular material are present in the phloem region. Cambium is present in 2-3 layered of tangentially elongated thin walled cells.

1.4.2.5Chemical Constituents

Several chemical constituents have been isolated from the *Ficus racemosa* plant. The stem bark showed the presence of two leucoanthocyanins: leucocyanidin-3-O-β-glucopyranoside, leucopelarogonidin-3-O-α-Lrhamnopyranoside, β- sitosterol, unidentified long chain ketone, ceryl behenate, lupeol, α- amyrin acetate. From trunk bark, lupeol, β-sitosterol and stigmasterol were isolated. Fruit of *Ficua racemosa* Linn. contains glauanol, hentriacontane, β- sitosterol, gluanol acetate, glucose, tiglic acid, and esters of taraxasterol, lupeol acetate, friedelin, higher hydrocarbons and other phytosterol. A new tetracyclic triterpene glauanol acetate which is characterized as 13α, 14β, 17 βH, 20 αH-lanosta-8, 22-diene-3 β-acetate and racemosic acid were isolated from the leaves. A thermo stable aspartic protease was isolated from the latex of the plant. The stem bark and fruit also showed presence of glauanol acetate. The leaf of this plant also contains sterols, triterpenoids in Petroleum ether extract and alkaloids, tannins and flavonoids in ethanolic extract.

1.4.2.6 Elemental Composition

The mineral composition of the bark is shown in Table. It is observed that potassium was the most abundant mineral present in the bark followed by chloride and calcium. The bark was a good source of iron, magnesium, phosphorous as well as trace elements such as manganese, nickel, chromium, zinc and copper. However, the bark contained significantly less sodium in proportion to potassium. The trace elements such as cadmium, aluminum, cobalt, mercury and arsenic were not detected.

Table 1.4: Concentration of mineral elements (±SD) in the bark of *Ficus racemosa* Linn.

Serial no:	Mineral elements	Concentrations (ppm)
01	Calcium	1729.3 ± 13.02
02	Iron	159.2 ± 2.03
03	Magnesium	196.2 ± 4.63
04	Phosphorous	443 ± 8.98
05	Zinc	0.49
06	Manganese	1.9 ± 0.14
07	Nickel	1.9 ± 0.14
08	Cadmium	ND
09	Chromium	0.38
10	Copper	5.2 ± 0.15
11	Lead	0.017 ± 0.003
12	Sodium	255 ± 42.03
13	Potassium	11975 ± 537.74
14	Chloride	7475 ± 263
15	Aluminium	ND
16	Cobalt	ND
17	Mercury	ND

1.4.3 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.

a) **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.

b) 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.

c) 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.

d) 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.

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

Chapter: 2

LITERATURE REVIEW

2.1 Phytochemical Studies

The leaf of this plant contains sterols, triterpenoids(Lanosterol) and alkaloids, tannins and flavonoids. Stem-bark gives gluanol acetate, β-sitosterol,leucocyanidin-3-O-β-D glucopyrancoside, leucopelargonidin-3-O-β-D-glucopyranoside, leucopelargonidin-3-O-α-L-rhamnopyranoside, lupeol, cerylbehenate, lupeol acetate and α-amyrinacetate. From trunk bark, lupenol, β-sistosterol and stigmasterol were isolated. Fruit containsgluanol acetate, glucose, triglicacid , esters oftaraxasterol, lupeol acetate, friedelin, higher hydrocarbons (Hentriacontane) and other phytosterols. A new tetracyclic triterpeneglauanolacetate which is characterized as 13α, 14β, 17βH,20αH-lanosta-8, 22-diene-3β-acetate and racemosicacid were isolated from the leaves. An unusualthermo-stable aspartic protease was isolated froml atex of the plant. The stem bark and fruit showedpresence of gluanol acetate (Joseph B*et al* ,. 2010).

2.2 Pharmacological Studies

2.2.1 Anti-diuretic:

The decoction of the bark of *F. racemosa* 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 h), peaked at 3 h and lasted throughout the study period (5 h). It also caused a reduction in urinary Na+ level and Na+/K+ ratio, and an increase in urinary osmolarity indicating multiple mechanisms of action (Ratnasooriya*et al*,. 2003).

2.2.2 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 (Bhaskara*et al*,. 2003).

2.2.3. Anthelmintic:

The crude extracts of bark were evaluated for anthelmintic activity using adult earthworms; they exhibited a dose-dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick, which was comparable witht hat of 3% piperazine citrate. However, there wasno

final recovery in the case of worms treated with aqueous extract suggesting wormicidal activity (Chandrashekhar*et al.*,2008).

2.2.4. Antibacterial:

The hydro alcoholic extract of leaves was found effective against *Actinomyces vicosus*. The minimum inhibitory concentration was found to be0.08mg/ml (Shaikh T*et al*,. 2010).

2.2.5 .**Antipyrectic:**

Methanol extract of stem bark was evaluated onnormal body temperature and yeast-induced pyrexia in albino rats, at doses of 100, 200 and 300mg/kg body wt. p.o. It showed significant dose dependent reduction in normal body temperature nd yeast-provoked elevated temperature which extended up to 5 hafter drug administration. Theanti-pyretic effect was comparable to that ofparacetamol (Rao RB *et al.*,2002).

2.2.6. Wound healing:

Ethanol extract of stem bark showed wound healing in excised and incised wound model in rats (Biswas*et al.*, 2003).

2.2.7. Antifilarial:

Alcoholic as well as aqueous extracts caused inhibition of spontaneous motility of whole worm and nerve muscle preparation of *Setariacervi*c haracterized by increase in amplitude and tone of contractions. Both extracts caused death of microfilariae *in vitro*. LC50 and LC90 were 21 and 35 ng/ml, respectively for alcoholic, which were 27and 42 mg/ml for aqueous extracts (Mishra V*etal*,. 2005).

2.2.8. Antidiarrhoeal:

Ethanol extract of stem bark was evaluated for antidiarrhoealactivity against different experimental models of diarrhoea in rats. It showed significant inhibitory activity against castor oil induced diarrhoea and PGE2 induced enteropooling in rats. These extracts also showed a

significant reduction in gastrointestinal motility in charcoal meal tests in rats. The results obtained established its efficacy as anti-diarrhoeal agent (Mukherjee PK*et al*,. 1998).

2.2.9. Anti-inflammatory:

The anti-inflammatory activity of *F. racemosa*extract was evaluated on carrageenin, serotonin,histamine and dextran-induced rat hind paw edemamodels. The extract (400 mg/kg) exhibitedmaximum anti-inflammatory effect of 30.4, 32.2, 33.9 and 32.0% with carrageenin, serotonin, histamine, dextran-induced rat paw edema, 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 IC50 values of 90 and 18 μM, respectively.Ethanol extract of stem bark also inhibited COX-1 with IC50 value of 100 mg/ml proves that the drug is used in the treatment of inflammatory conditions (Li RW *et al*,. 2004).

2.2.10. Antiulcer:

The 50 % ethanol extract of fruits was studied in different gastric ulcer models, viz pylorus ligation, ethanol and cold restraint stress induced ulcers in rats at a dose of 50, 100 and 200 mg/kg body weight p.o. for 5 days twice daily. The extract showed dose dependent inhibition of ulcer index in all three models of ulcer (Patel SM *et al*,.1985).

2.2.11. Analgesic:

The ethanol extract of bark and leaves was evaluated for analgesic activity by analgesiometer at 100, 300 and 500 mg/kg and was found to possess dose dependent analgesic activity (Malairajan P*et al* ,. 2006).

2.2.12. Hepatoprotective:

An ethanolic extract of the leaves was evaluated for hepatoprotective activity in rats by inducing chronic liver damage by subcutaneous injection of 50% v/v carbon tetrachloride in liquid

paraffin at a dose of 3 mL/kg on alternate days for a period of 4 weeks. The biochemical parameters SGOT, SGPT, serum bilirubin and alkaline phosphates were estimated to assess the liver function. In other study, the methanol extract of stem bark at the doses of 250 and 500 mg/kg was evaluated for its hepatoprotective activity in rats against carbon tetrachloride induced liver damage with silymarin as standard. It showed significant reversal of all biochemical parameter towards normal when compared to carbon tetrachloride treated control rats in serum, liver and kidney (Channabasavaraj KP *et al* ,. 2008).

2.2.13. Radio protective/antioxidant:

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 FRE was studied using micronucleus assay in irradiated Chinese hamster lung fibroblast cells. Pretreatment with different doses of FRE 1h prior to 2 Gy γ-radiation resulted in a significant decreasein the percentage of micronucleated binuclear 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.Thefruit ethanol extract exhibited significant antioxidant activity in DPPH free radical scavenging assay. 3-*O*-(*E*)-Caffeoylquinate showed significant antioxidant activity (Veerapur VP*et al*,. 2009).

2.2.14. Antifungal:

The plant possesses potent inhibitory activity against six species of fungi, viz. *Trichophytonmentagrophytas, Trichophytonrubrum,Trichophytonsoundanense, Candida albicans,Candida krusei*and *Torulopsis glabrata.*(Deraniyagala SA et al,. 1998).

2.2.15. Hypoglycemic:

The glucose-lowering efficacy of methanol extract of the stem bark was evaluated both in normal andalloxan-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 anti-diabetic agent, glibenclamide (10 mg/kg) proving its folklore claim as anti-diabetic agent. The relationship of the post absorptive state to the hypoglycemic studies on *F. racemosa*showed that the absorption of the drug leads to a better hypoglycemic activity. The ethanol extract (250 mg/kg/day) lowered blood glucose level within 2 weeks in the alloxan diabetic albino rats confirming itshypoglycemic activity. β-sitosterol isolated from the stem bark was found to possess potent hypoglycemic activity when compared to other isolated compounds. 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.Ethanolic extract of leaves lowered the blood glucose levels by 18.4 and 17.0% at 5 and 24 h, respectively, in sucrose challenged streptozotocininduced diabetic rat model at the dose of 100 mg/kg body weight (*Sophia D et al*,.2007).

2.2.16. Hypolipidemic:

Dietary fiber content of fruits when fed to rats in diet induced pronounced hypocholesterolemiceffect, as it increased fecal excretion of cholesterol as well as bile acids .Hypolipidemic activities of ethanolicextract of bark were studied at the doses of 100-500 mg/kg 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 (Agarwal V *et al*,.1988).

1.4.17. Renal anticarcinogenic:

*F. racemosa*extract (200 mg/kg body weight and 400 mg/kg body weight) resulted in a significant decrease in xanthine oxidase, lipid peroxidation, γ- glutamyltranspeptidase and hydrogen peroxide. There was significant recovery of renal glutathionecontent and antioxidant enzymes, decrease in the enhancement of renal ornithine decarboxylaseactivity, DNA synthesis, blood urea nitrogen and serum creatinine. Similar results were obtained when Ferric nitrilotriacetate (Fe-NTA) was used as renal carcinogen. Both the results proved that the extract is a very potent chemopreventive agent (Khan N *et al*,. 2005).

1.4.18. Memory Enhancing

Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in dementia and enhancement of acetylcholine (Ach) levels in brain using acetyl cholinesterase inhibitors is one of the most important approaches for the treatment of AD. Aqueous extract of *Ficus racemosa* Linn bark having anti-inflammatory, antioxidant, and Anti-cholinesterase activity was evaluated for its ability to enhance Ach levels, and to ascertain its anti-dementia activity in rats. This work was carried out under the assumption that the *Ficus racemosa* Linn. extract may show combination of actions which could be beneficial in the treatment of AD, such as neuro protection, attributed to antioxidant and anti-inflammatory property and may elevate levels of Ach like *Ficus hispida* extract reported earlier. The plant extract selected for investigation elevated Ach levels and improved memory in rats. The collective pharmacological actions attributed by *Ficus racemosa* Linn extract may serve as beneficial and supporting agent in the treatment of AD (Mitra R *et al*,. 1985).

1.4.19. Anticholinesterase:

The various study evaluated the Anticholinesterase activity of cold and hot aqueous extracts of *Ficus racemosa* Linn stem bark against rat brain acetyl cholinesterase in vitro. Both the cold aqueous extract (FRC) and the hot aqueous extract (FRH) exhibited a dose dependent inhibition of rat brain acetyl cholinesterase. FRH showed significantly higher ($P \leq 0.001$) cholinesterase inhibitory activity compared to FRC; however, both the extracts did not show 50% inhibition of AChE at the doses tested (200-1000 µg/ml). The IC50 values of 1813 and 1331 µg ml−1 were deduced for FRC and FRH, respectively (calculated by extrapolation using Boltzmann's dose response analysis). Among FRC and FRH, FRH showed significantly higher ($P \leq 0.001$) cholinesterase inhibitory activity compared to FRC; however, both the extracts did not show 50% inhibition of AChE at the doses tested (200-1000 µg/ml) and hence IC50 values were calculated by extrapolationusing Boltzmann's dose response analysis(Joseph B *et al*,.2010).

1.4.20. Cardio-protective

Ficus racemosa Linn. bark is a rich source of phenolic compounds having diverse biological properties including antioxidant activity. Cardiotoxicity was induced by administration of

doxorubicin (10 mg kg (-1) i.v.) To the extract pretreated rats (250 and 500 mg kg (-1)) and compared with that of Arjuna, a standard cardiotonic.Biochemical parameters included CK-MB, LDH, AST, ALT, troponin I, thiobarbituric acid reactive substances (TBARS), and glutathione. The HPLC fingerprinting of the extract indicated the presence of bergenin (0.89%) and bergapten (0.07%). In an acute toxicity study, the extract at a dose of 2 g kg (-1) did not cause any adverse changes and no mortality was observed. Administration of doxorubicin significantly increased ($p \leq 0.05$) serum levels of creatine kinase,lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase, which were decreased to an extent of 68, 63, 41, and 65%, respectively, in extract pretreated group (500 mg kg(-1)). Troponin I was undetected in control group, while it was found in serum of all the experimental groups. The extract pretreatment significantly decreased ($p \leq 0.05$) TBARS and increased glutathione levels in serum and cardiac tissue. These observations were further substantiated by the histopathological studies. The acetone extract of *Ficus racemosa* Linn. Bark possesses potential cardio protective activity against doxorubicin-induced cardiotoxicity in rats by scavenging free radicals generated by the administration of the drug(Channabasavaraj KP*et al*,.2008).

1.4.21. Gastroprotective

Gastroprotective effect of 50% ethanolic extract of *Ficus racemosa* Linn. fruit was studied in different gastric ulcer models in rats.ods: (50, 100 and 200 mg/kg body weight) was administered orally, twice daily for 5 days for prevention from pylorus ligation (PL), ethanol (EtOH) and cold restraint stress (CRS) induced ulcers. Estimation of H+K+ATPase activity and gastric wall mucous were performed in EtOH-induced ulcer and antioxidant enzyme activities in supernatant mitochondrial fraction of CRSinduced ulcers. FRE showed dose dependent inhibition of ulcer index in pylorus ligation, ethanol and cold restraint stress – induced ulcers. FRE 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. High performance thin layer chromatography (HPTLC) analysis showed the presence of 0.57% and 0.36% w/w of gallic acid and ellagic acid in FGE(Rao CHV *et al*,. 2008).

1.4.22. 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 activity, 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 (Li RW *et al*,. 2003).

1.4.23. Treatment of cancer

Medicinal plant products exhibiting anticancer activity continue to be the subject of extensive research aimed at the development of new or alternative drugs for the treatment of different human tumors.It is suggested that *F. glomerata* and *Ficus racemosa* Linn used for the treatment of skin cancer [36]. Both the natural and compounds synthesised from F. carica showed in vitro inhibitory effects on proliferation of various cancer cell lines. Fruit extracts of *F. benjaminaLinn, F. bengalensis , F. religiosa Linn* and *Ficus sycomorus Linn*., an African species, exhibited anti tumor activity in the potato disc bioassay (Bhatt RM *et al* ,.1984).

Chapter: 3

METHODS & MATERIALS

3.1. Collection & Preparation Of Plant Material

Plant sample (Leaves) of *Ficus racemosa* was collected from Gopalgonj 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 DCM

The mother solution was taken in a separating funnel. 100ml of the Dichloromethane (DCM) 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 DCM fraction was then air dried for solid residue.

3.4.2 Partition With Petroleum Ether

To the mother solution left after partitioning with DCM, 12.5ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with petroleum ether 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 washing with DCM and petroleum ether, 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 Aquous

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

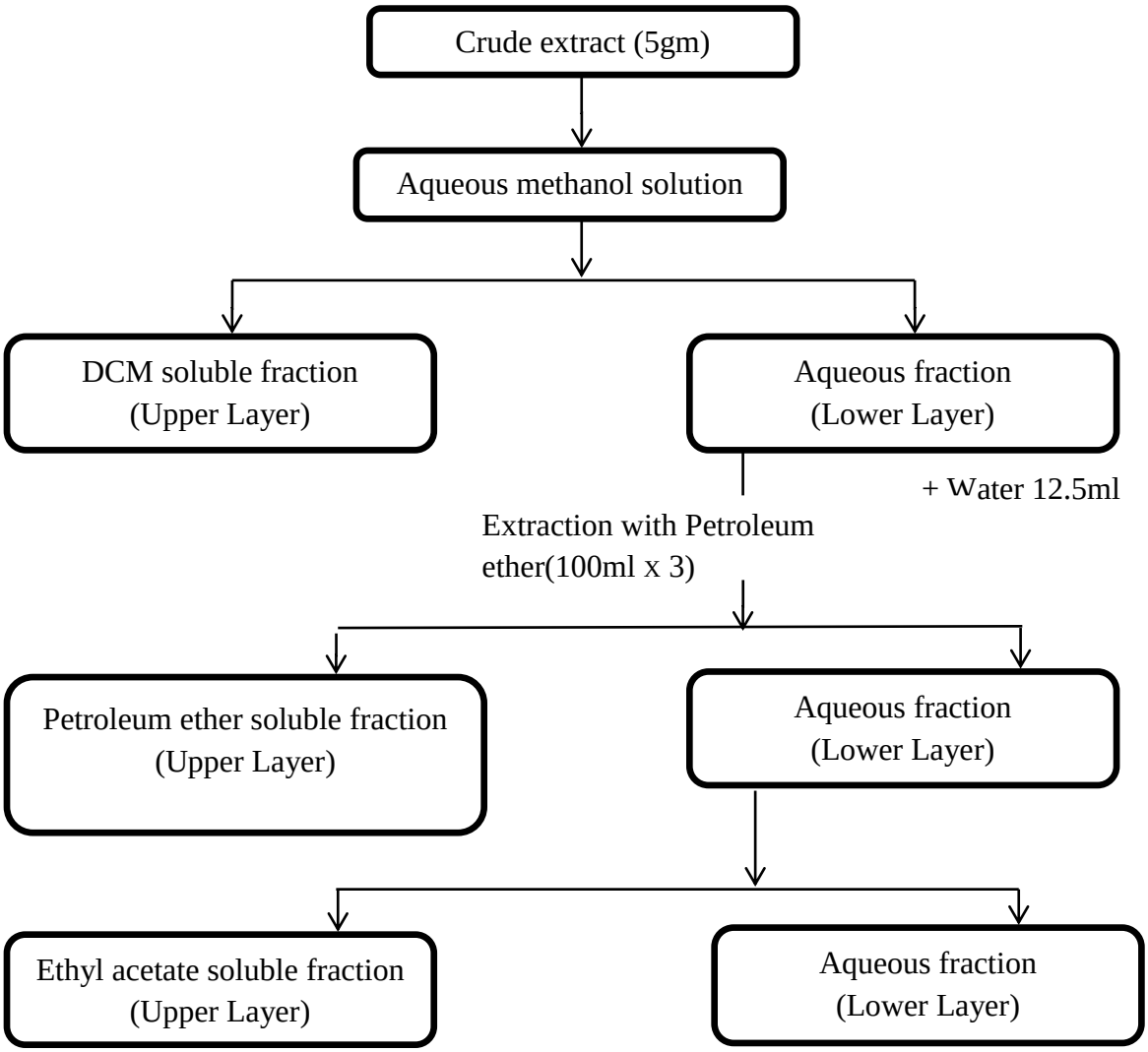


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Ficus racemosa* leaves

\3.5 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 *Artemiasalina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemiasalina* were hatched at room temperature (25-30ºC) for 18-24hr. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay.

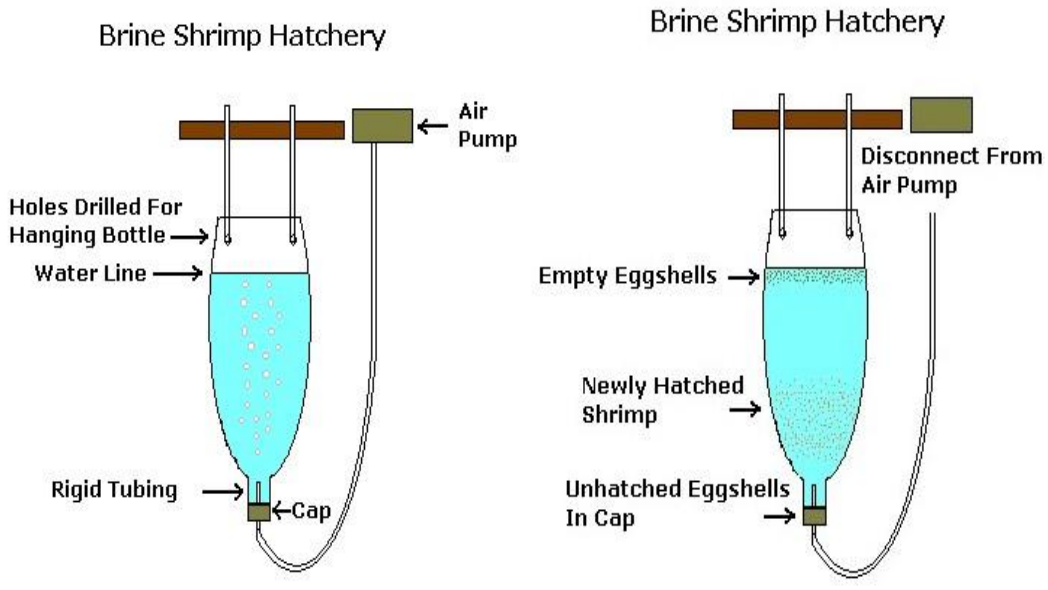


Figure 3.3:Brine Shrimp Hatchery

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).

3.6.2Apparatus &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 racemosa*

Petroleum ether fraction of methanolic fraction of *Ficus racemosa* 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* *Sarcina lutea* *Staphylococcus aureaus*
Gram –ve	*Escherichia coli* *Salmonella paratyphi* *Shigella dysenteriae* *Vibrio parahaemolyticus*
Fungi	*Aspergillus niger* *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.

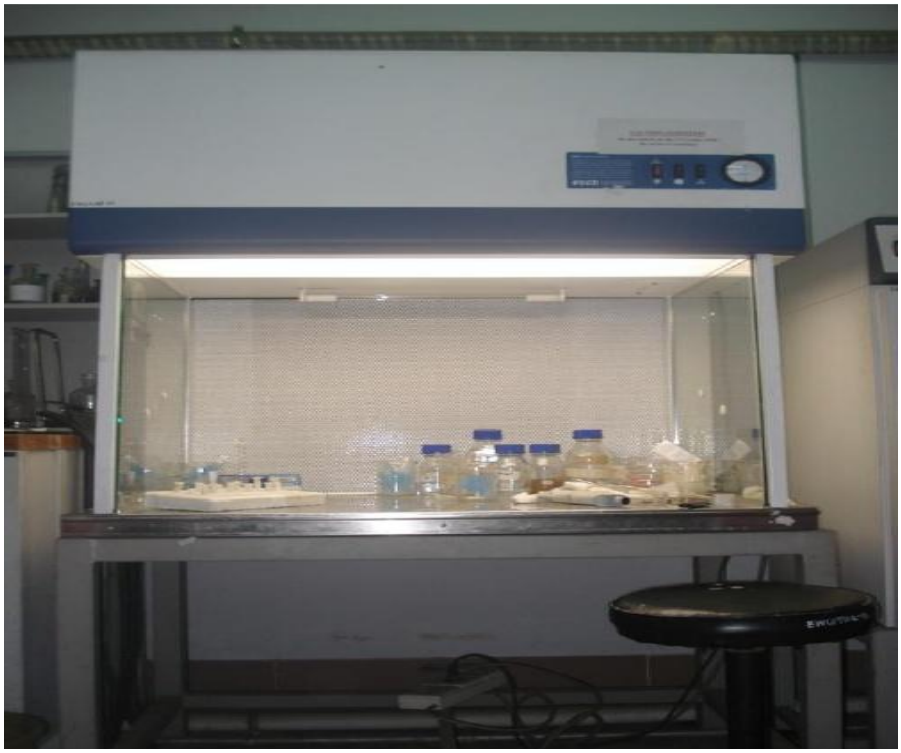


Figure 3.5: Laminar hood

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.

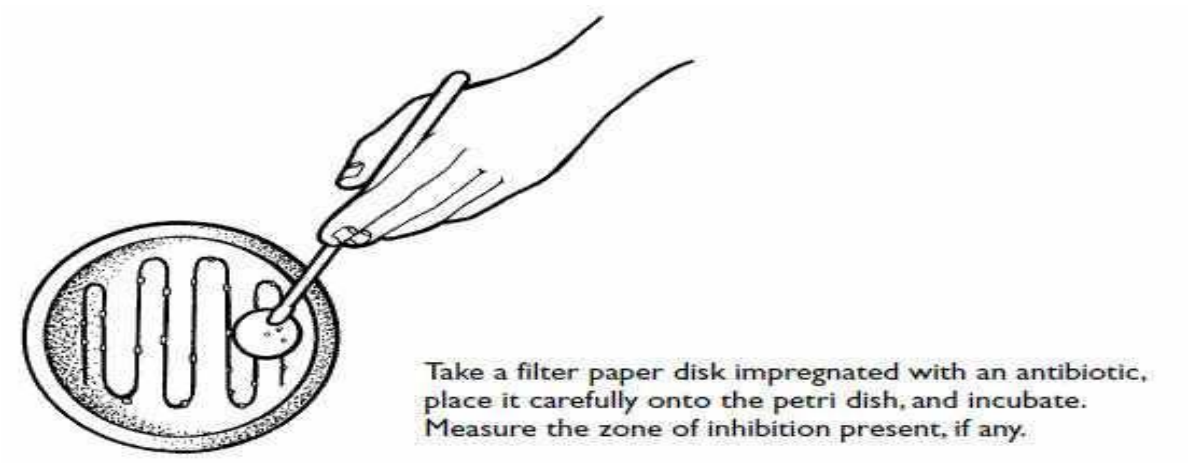


Figure 3.6: Preparation of filter paper discs

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

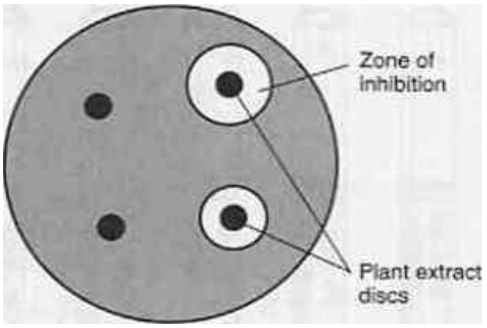
3.6.5.7 Diffusion & Incubation

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.



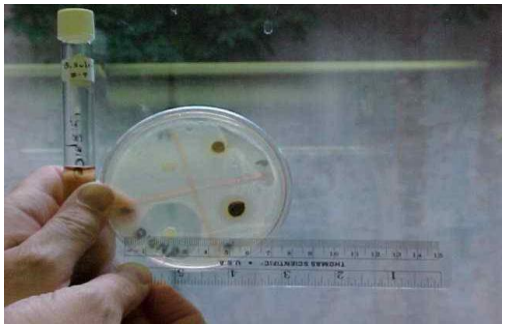


Figure 3.8: Clear zone of inhibition **Figure 3.9**: Determination of clear zone of inhibition

3.7 Antioxidant Activity

3.7.1 Total Phenolic Content

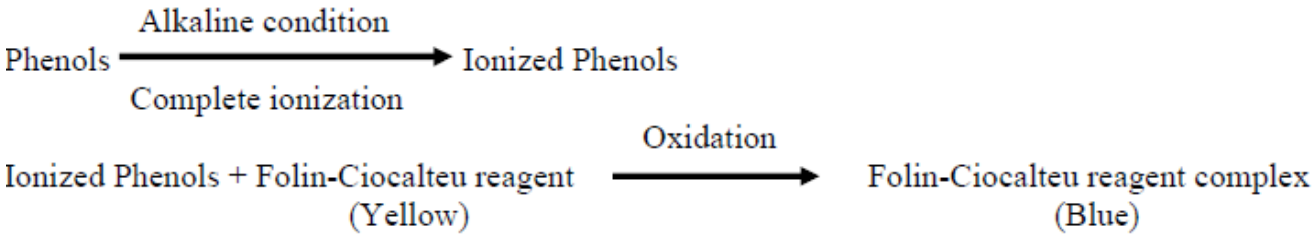
3.7.1.1 Principle

The content of total phenolic compounds in plant 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 3.6: 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* petroleum ether 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 3.7: 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 3.8: 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.

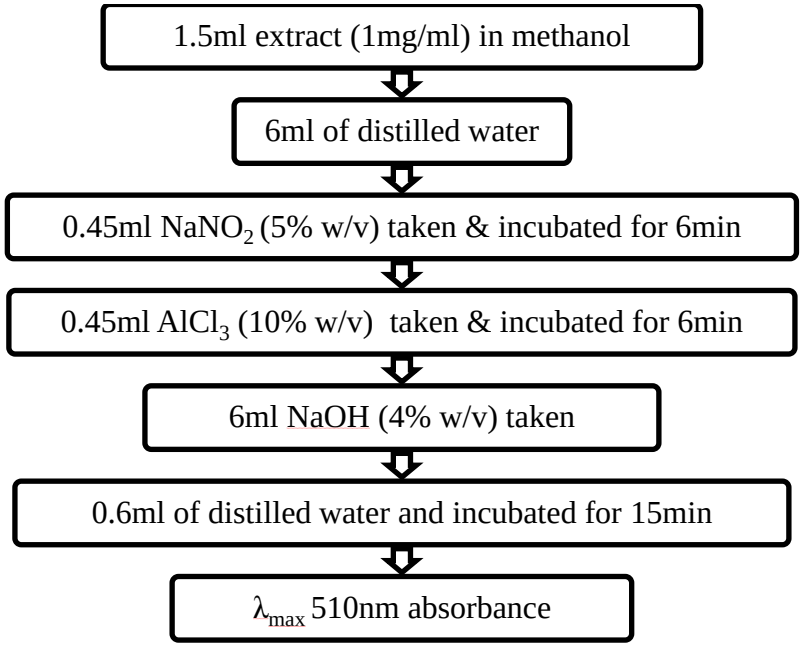


Figure 3.10: Schematic diagram of preparation of extract solution

Preparation of blank solution:

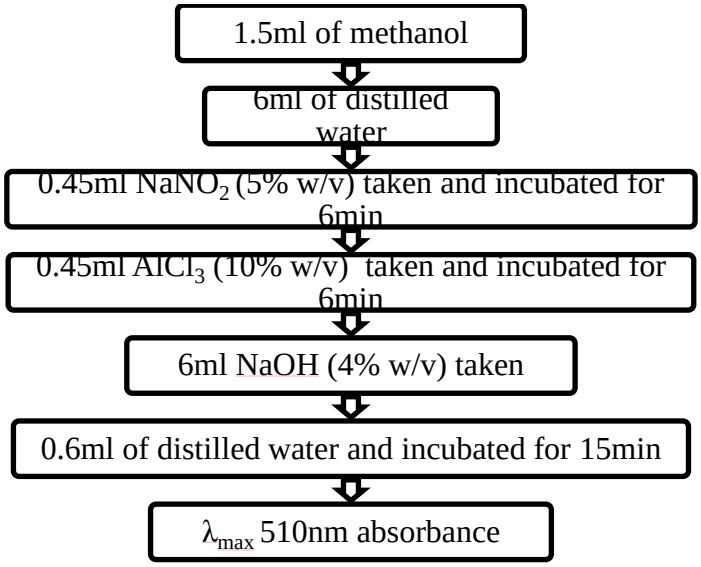


Figure 3.11: Schematic diagram of preparation of blank solution

Chapter: 4

RESULTS & DISCUSSION

4.1 Result Of Brine Shrimp Lethality Bio-Assay

The petroleum ether fraction of the *Ficus racemosa* 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 ethyl acetate extract that produces death in half of the test subjects after a certain period. The percentage mortality at each concentration was determined using the following formula:

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

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

4.1.1 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	

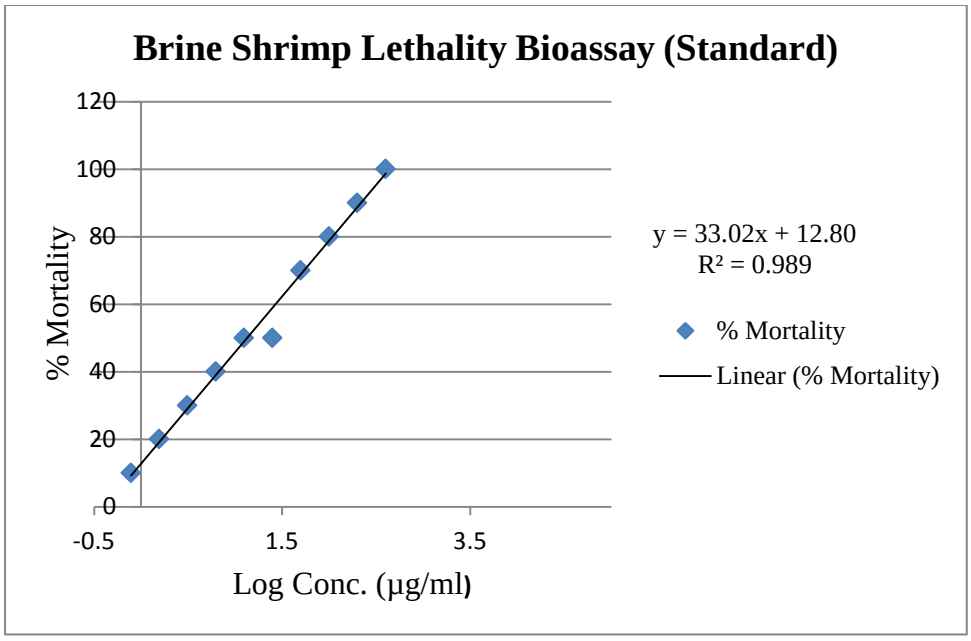


Figure 4.1: % mortality and predicted regression line of Tamoxifen (standard)

4.1.2 Preparation Of Petroleum Ether Fraction Curve

Table 4.2: Results of the bioassay of petroleum ether 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	22.9
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	4	6	60	
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	6	4	40	
9	1.5625	0.194	7	3	30	
10	0.78125	-0.107	7	3	30	

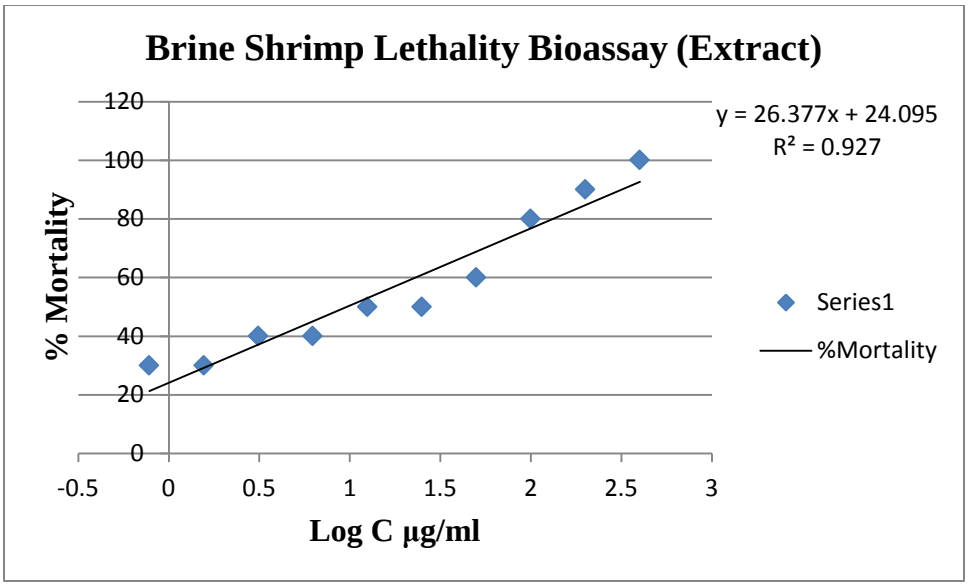


Figure 4.2: % Mortality and predicted regression line of petroleum ether fraction (extract)

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 ethyl acetate 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 Petroleum ether fraction of *Ficus racemosa* 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 (Petroleim ether fraction)	y = 26.37x + 24.09	0.927	22.9

In this investigation, standard and petroleum ether fraction exhibited cytotoxic activities with the LC_{50} values 13.38µg/ml and 22.9µg/ml respectively as shown in Table 4.3. For Petroleum ether fraction R^2 value is less than the standard which indicates that the extract has less potent activity than standard against brine shrimp nauplii.

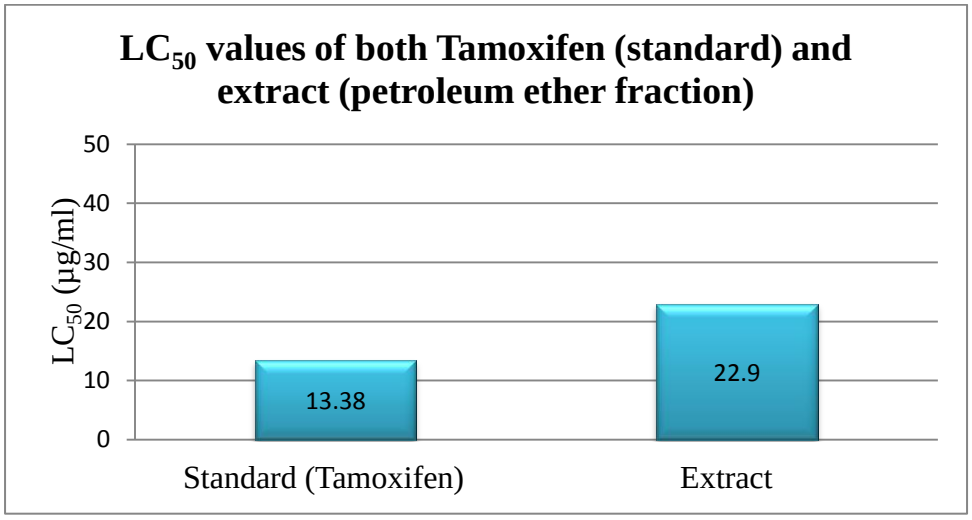


Figure 4.3: Comparison between LC_{50} values of standard and extract

4.2 Result Of Antimicrobial Test

The antimicrobial activities of petroleum ether fraction of *Ficus racemosa* 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 Petroleum Ether Fraction

Table 4.4: Antimicrobial activity of standard sample(Ciprofloxacin) and petroleum ether fraction

Type of microorganism		**Zone of inhibition (mm)**	
		Standard sample	**Petroleum ether fraction**
Gram positive bacteria	*Bacillus cereus*	18	12
	Bacillus subtilis	20	12
	Sarcina lutea	18	12
	Staphyllococcus aureaus	20	8
Gram negative bacteria	*Escherichia coli*	18	9
	Pseudomonas aeruginosa	25	8
	Salmonella paratyphi	20	4
	Shigella dysenteriae	18	12
Fungi	*Candida albicans*	18	8

4.2.2 Discussion

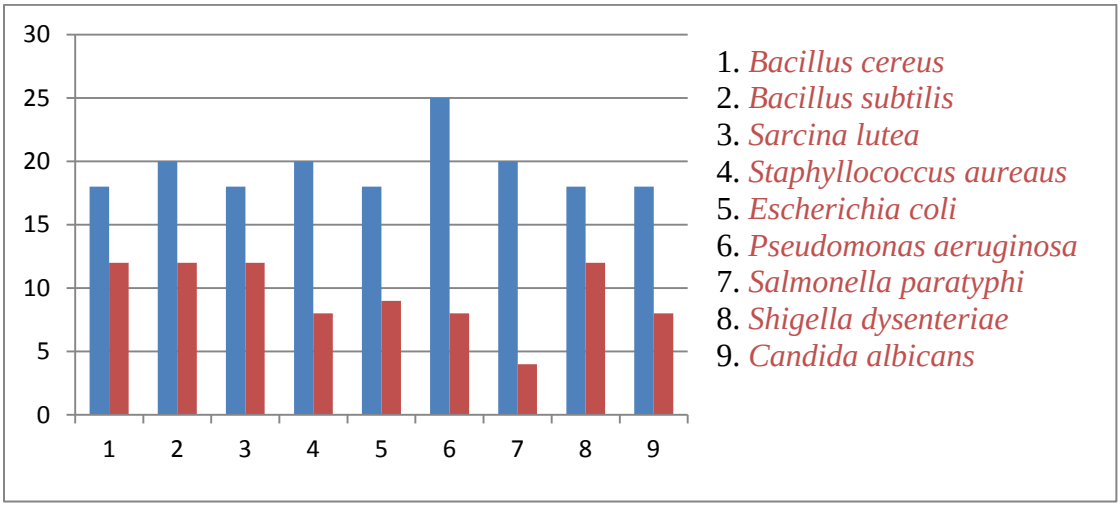


Figure 4.4: Comparison of antimicrobial activity between standard and extract

Petroleum ether fraction of *Ficus racemosa* leaves extract showed low to moderate antimicrobial activity when compared to reference standard drug Ciprofloxacin. None of the zone of inhibition of ethyl acetate fraction is equal to Ciprofloxacin against any bacteria or fungi as shown in the Figure: 4.4. Among all the microbiological cultures, the fraction showed the best antimicrobial activity against*Beta-hemolytic streptococcus* (12mm)&*Pseudomonas aeruginosa* (12mm) comparable to the standard (18mm).

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 petroleum ether fraction of*Ficus racemosa* leaves extract was determined byfollowing methods-

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

4.3.1 Result Of Total Phenolic Content

The petroleum ether 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.

4.3.1.1 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 Petroleum ether fraction of *Ficus racemosa* leaves extract

Sample	Concentration (mg/ml)	Absorbance	Average X value (mg of AAE/gmof dried extract)
Petroleum ether fractionof *Ficus Recemosa*	2	0.473	40.01

4.3.1.2 Discussion

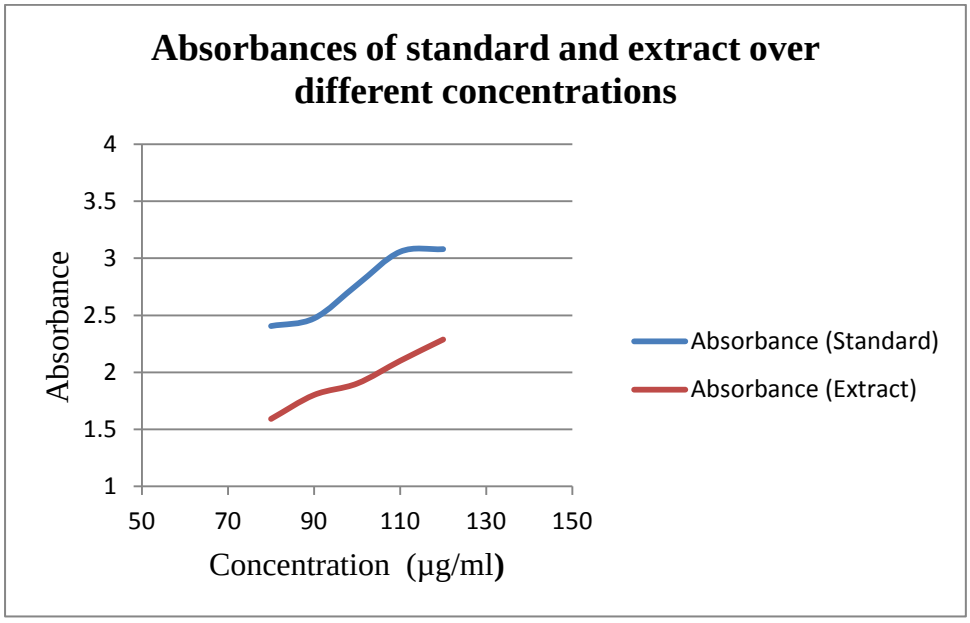


Figure 4.5: Comparison between absorbances of standard and extract over different concentrations

The absorbance was found to be directly proportional to the concentration in both standard and petroleum ether fraction samples. In both extract and 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, 40.01mg of AAE/gm of dried extract of phenol content was found in the petroleum ether fraction of *Ficus racemosa* leaves.

4.3.2 Result Of Total Flavonoid Content

Petroleum ether fraction of *Ficus racemosa* leaves were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard.

4.3.2.1 Preparation Of Standard Curve

Table 4.6: 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 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.

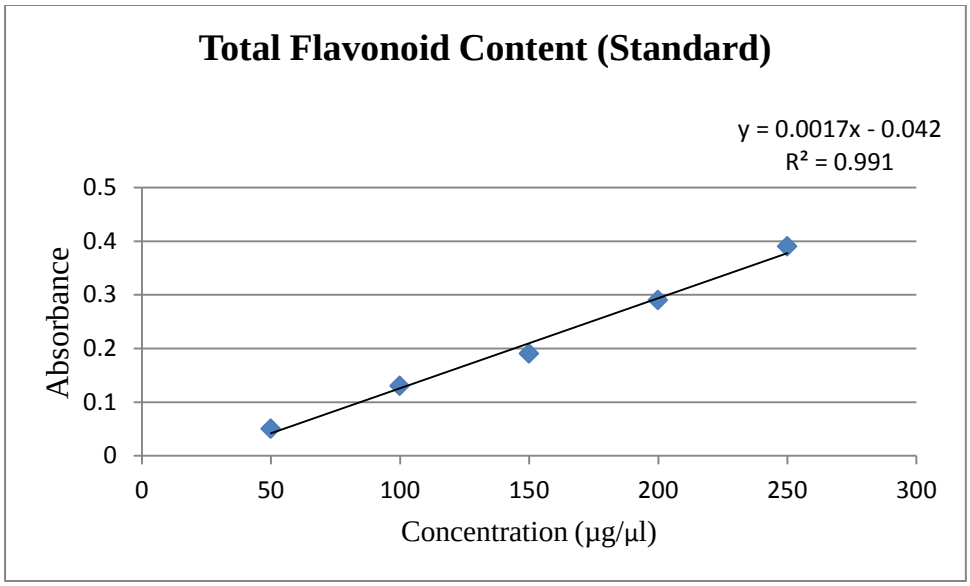


Figure 4.6: Graphical representation of assay of flavonoid content of ascorbic acid

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 4.7.

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

Sample	Concentration (mg/ml)	Absorbance	Total flavonoid content (mg of AAE/g of dried extract)
Petroleum ether fraction of *Ficus racemosa*	1	0.644	435

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 ethyl acetate fraction of *Ficus racemosa* (leaves) 435mg 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

As the literature review suggests, the presence of several phytochemical compounds in *F. racemosa* makes the plant pharmacologically active. The present study showed that it has very good antioxidant activity that could make it a potent drug against free radical mediated diseases. The petroleum ether extract possesses cytotoxic activity that could be a better treatment in tumor as well as cancer. The study also showed that, the extract showed low to moderate antimicrobial activity that could be a better treatment in antimicrobial infections. However, studies are required on higher animal model and subsequently on human subjects to prove efficacy as an antioxidant, cytotoxic and antimicrobial agent.

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

Chapter: 6

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