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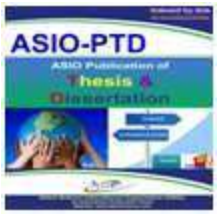

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PHYTOCHEMICAL SCREENING & BIOLOGICAL INVESTIGATIONS OF FICUS RACEMOSA

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B. PHARM. THESIS

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B. PHARM. THESIS

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Ficus racemosa is an important medicinal plant, found in India, Australia, and Southeast Asia. It is popularly known as 'gular.' It reduces blood glucose concentration due to the presence of β-sitosterol. Many active constituents that have been isolated from various parts of this plant possess useful pharmacological activities. The objective of this dissertation is to identify the biological activity of the roots of an indigenous medicinal plant, viz., Ficus racemosa (Family: Moraceae) and to evaluate the possible phytochemical and pharmacological profiles of the crude extracts. So far, some chemical and biological investigations have been carried out on this plant mainly focusing on the bark and root of the plant. That's why the goal of this framework is to explore the potential possibilities of developing new drug candidates from the fruit of this plant which could be crucial for the treatment of various ailments.

Phytochemical Screening & Biological Investigations of *Ficus racemosa*

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Abstract

Ficus racemosa is an important medicinal plant, found in India, Australia, and Southeast Asia. It is popularly known as 'gular.' It reduces blood glucose concentration due to the presence of β-sitosterol. Many active constituents that have been isolated from various parts of this plant possess useful pharmacological activities. The objective of this dissertation is to identify the biological activity of the roots of an indigenous medicinal plant, viz., Ficus racemosa (Family: Moraceae) and to evaluate the possible phytochemical and pharmacological profiles of the crude extracts. So far, some chemical and biological investigations have been carried out on this plant mainly focusing on the bark and root of the plant. That's why the goal of this framework is to explore the potential possibilities of developing new drug candidates from the fruit of this plant which could be crucial for the treatment of various ailments.

Introduction:

1.1 Rationale and Objective of the Work

Phytochemistry is a branch of Pharmacognosy, with chemical and biological characters, which study the obtaining of medicaments by natural or semi synthesis methods? The subject "Phytochemstry" deals with the chemical structures of secondary metabolites, their metabolism, and their distribution and biological functions [1].

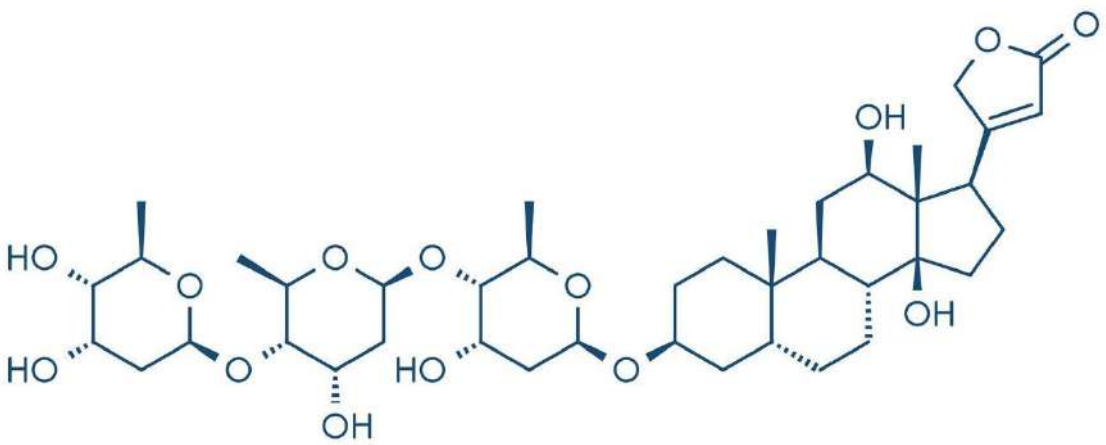
Written records of the use of plants as medicinal agents date back thousands of years. The oldest records come from Mesopotamia and date from about 2600 BC. Those records are not simply a case of one or two plant based 'drugs' finding their way into popular use, because the documents indicate that there were many drugs in use that contained plants (up to 1,000 in the case of Mesopotamia) [2].

Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world. In most of the traditional, the medicinal plants include the fresh or dried parts, whole, chopped, powdered or an advanced form of the herb usually made via extraction by a solvent such as water, ethanol or an organic solvent play a major role and constitute the backbone of traditional medicine[3].

Goals of using plants as sources of therapeutic agents are:
To isolate bioactive compounds for direct use as drugs e.g. digoxin, digitoxin, morphine, reserpine, taxol, vincristine, vinblastine.

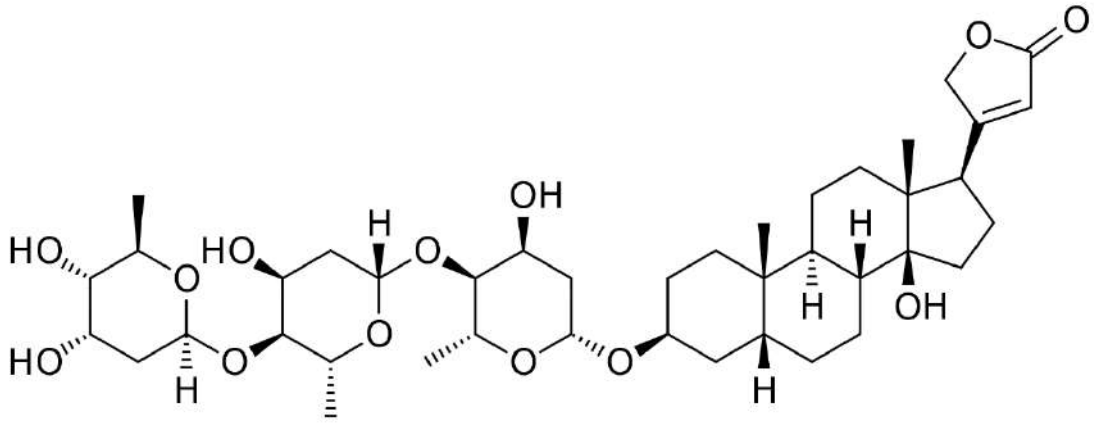
1. To produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher activity and/or lower toxicity e.g. metformine, nabilone, oxycodone, taxotere, teniposide, verapamil and miodarone which are based on galegine, A9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin, khellin.
2. To use agents as pharmacologic tools e.g. lysergic acid diethylamide, mescaline [4].

There are several familiar approaches for lead searching from the plants and the isolated bioactive compounds are utilized in three basic ways:
Unmodified medicinal plant products where ethnomedical uses suggested clinical efficacy e.g. digitoxin (a), digoxin (b), morphine (c).

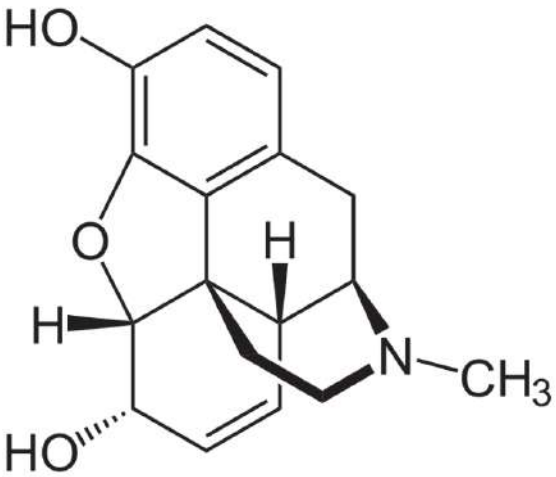


(a)

Digoxin (source: Chemistry World)



(b) Digitoxin (source: sciencedirect.com)



(c) Morphin (source: ChemSpider)

Figure 1.1: Lead compound search & utilization from plants.

1. Unmodified natural products of which the therapeutic efficacy was only remotely suggested by indigenous plant use e.g. vincristine.
2. Modified natural or synthetic substances based on a natural product used in folk medicine e.g. aspirin [5].
3. Terrestrial plants, especially higher plants, have a long history of use in the treatment of human diseases [6]. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago[7].Several well-known species, including licorice *(Glycyrrhiza glabra)* myrrh *(Commiphora*species), and poppy capsule latex *(Papaver somnjferum),* were referred to by the first known written record on clay tablets from Mesopotamia in 2600 BC, and these plants are still in use today for the treatment of various diseases as ingredients of official drugs or herbal preparations used in systems of traditional medicine. Furthermore, morphine, codeine, noscapine (narcotine), and papaverine isolated from *P. somnferum*were developed as single chemical drugs and are still clinically used. Hemisuccinate carbenoxolone sodium, a semi-synthetic derivative of glycyrrhetic acid found in licorice, is prescribed for the treatment of gastric and duodenal ulcers in various countries [6].

Success in natural products research is conditioned a careful plant selection, based on various criteria such as chemotaxonomic data, ethnomedical information, field observations or even

random collection [8]. Historical experiences with plants as therapeutic tools have helped to introduce single chemical entities in modem medicine. Plants, especially those with ethno-pharmacological have been the primary sources of medicines for early drug discovery. In fact, a recent sis by Fabrican and Farnsworth showed that the uses of 80% of 122 plant-derived drugs were related to their original ethno pharmacological purposes. Current drug discovery from terrestrial plants has mainly relied in bioactivity-guided isolation methods, which, for example, have led to discoveries of the important anticancer agents, paclitaxel from *Taxus brevifolia*and camptothecin from *Camptothecaacuminate* [6].

The goals of using plants as sources of therapeutic agents are a) to isolate bioactive compounds for direct use as drugs, e.g. digoxin, digitoxin, morphine, reserpine, taxol, vinbiastine, vincristine; b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere, teniposide, verapamil, and miodarone, which are based, respectively, on galegine, Δ9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin, and khellin; c) to use agents as pharmacologic tools, e.g., lysergic acid diethlamide (LSD), mescaline, yohimbine; and d) to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, etc[9].The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000[10].with a lower level at 215,000 [11].and an upper level as high as 500,000[12].Of these, only about 6% have been screened for biologic activity, and a reported 5% have been evaluated phytochemically[13].It was estimated that in 1991 in the United States, for every 10,000 pure compounds most likely those based on synthesis) that arebiologically evaluated (primarily in vitro), 20 would be tested in animal models, and 10 of these would be clinically evaluated, and only one would reach U.S. Food and Drug administration approval for marketing. The time required for this process was estimated at 10 years at a cost of $231 million (U.S.) [14].

The major drawback of this strategy is the frequent isolation of known metabolites. Therefore, hyphenated techniques (LC-UV, LC-MS, and LC-NMR have been developed; in order to detect is early as possible potential original structures. These compounds can then be tested in various bioassays [8]. More recently combinatorial chemistry and high throughput robotic screening techniques have been employed as viable strategies for drug discovery programs [15]. Chemical diversity of secondary plant metabolites that results from plant evolution is superior to at found in synthetic combinatorial chemical libraries [14]. Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. (E.g. *Dutura* has long been associated with the worship of Shiva, the Indian God). 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. Studying medicinal plants helps to understand the 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[16].The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases. In real sense, coupling of ancient knowledge and scientific principle is essential- (1) to identify alternative and complementary medicine. (2) To reduce the toxicity of drug therapy especially toxicity reduction of synthetic

and semi synthetic drugs. (3) To find the lead compound diversification to treat various diseases [17].

1.2 History of traditional herbal medicine in Bangladesh

Traditional Medicine is the medicine or treatment based on traditional uses of plants, animals or their products, other natural substances (including some inorganic chemicals), religious verses, cultural practices, and physical manipulations including torture. As this system of medicine has been in use almost unchanged generation after generation throughout the ages for the treatment of various physical and psychological diseases, it is called traditional. Most of the times, the type, preparation, and uses of traditional medicines are largely influenced by folklore customs and the cultural habits, social practices, religious beliefs and, in many cases, superstitions of the people who prescribe or use them[18].The earliest mention of traditional medicine is found in *"Rigveda",* the oldest repository of knowledge in this subcontinent. Later *"Ayurveda",* developed from the *Vedic* concept of life, became the important source of all systems of medical sciences. In course of time it became a part of culture and heritage of the people of the Indian subcontinent Traditional medicine involves the use of both material and non-material components. The material components invariably comprise parts or organs of plants and their products. They also consist of animal organs, minerals and other natural substances. The non-material components, which constitute important items of religious and spiritual medicines, include torture, charms, magic, incantations, religious verses, amulets and rituals like sacrifices, appeasement of evil spirits, etc. [18].Treatments in traditional medicine are carried out by internal and external application of medicaments, physical manipulation of various parts of the body, performing rituals, psychological treatment, and also by minor surgery. Ayurvedic medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances, minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in Ayurvedic medicine[18].Whole plants or their powders or pastes or products and their extracts, infusions, decoctions and distillates constitute the major constituents of Unani medicine. Minerals, inorganic chemicals and animal products are also frequently used in preparing these medicines. For hundreds of years, the medical knowledge of the Indian subcontinent is termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India and Bangladesh. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Toda' the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field[19].Some crude drugs used as medicine in Bangladesh are reported in following table.

Table 1.1 Some crude drugs used as medicine Bangladesh [20]

Plant	Biological Source	Plant part in use	Important content	Use
Punarnava	*Boerhaavia diffusa*	Root	Alkaloids, Xanthenes, Ursolic acid	Diuretic, useful in nephritic syndrome, chronic edema and liver diseases
Vasaka	*Adhatoda vaska*	Dried/fresh leaves	Vasicine, Vasicinone (alkaloid)	Cough & cold, chronic bronchitis & asthma, expectorant
Anantamul	*Hemidesmus indicus*	Root	Essential Oil, Saponin, Resin, Tannins, Sterols and glucosides.	Tonic, diuretic, demulcent, diaphoretic, carminative
Arjun	*Tarminalia arjuna*	Leaves & bark	Tannins, β-sitosterols, saponin	As cardio tonic in angina pain, diuretic in palpitations
Chirata	*Gentiana chirayita*	Entire dried plant	Gentiopicrin (bitter glycoside)	Bitter tonic, febrifuge, stomachic & laxative
Picrorhiga	*Picrirhiga kurroa*	Dried rizomes	Picrorhigin (Glycoside)	Bitter tonic, cathartic, stomachic used in dyspepsia, anti-periodic & colagogue
Kalomegh	*Andrographis paniculata*	Leaves or entire aerial part	Kalmeghin (bitter crystalline diterpin lactone)	Febrifuge, astringent, anthelmintic. Useful in cholera, piles, gonorrhea, dyspepsia and general weakness
Amla (Triphala)	*Phylanthus emblica*	Dried fruit	Vit C (20 times more than in orange)	Cooling, refrigerant, diuretic & laxative, promotes hair growth
Bahera	*Terminalia belerica*	Dried ripe fruit	20% tannins, phyllembin, mannitol	Bitter tonic, astringent, laxative, antipyretic used in dysentery, piles, leprosy
Haritaki	*Terminalia Chebula*	Fruit	Triterpenes & conjugated coumarins	Carminative, appetite stimulant used in leprosy, anemia, piles, intermittent fever, heart disease, diarrhea
Tulsi	*Ocimum sanctum*	Leaves	Eugenol (essential oil), carvacrol	Expectorant, diaphoretic, antiperiodic, antiseptic & spasmolytic
Neem	*Azadiachta indica*	Leaves & seed oil	Nimbin, nimbinene, nimbandiol (indole alkaloids)	Stimulant, antiseptic used in rheumatism & skin diseases
Garlic	*Alium sativum*	Bulb	Designated allicin	Used in hypertension, stimulating bile production, common cold, acceleration in wound healing
Spirulina	*Spirulina maxima*	Blue-green algae	Protein and Vit B_{12}	Weight loss
Ginseng	*Panax quinquefolius*	Root	Complex mixture of triterpenoid saponins	Aphrodisiac
Aloe	*Aloe barbadensis*	Dried latex juice of leaves	Barbaloin (anthraquinine glycosides)	Benzoin tincture, cathartic

1.3 Research of traditional drugs in Bangladesh

In rural areas medicinal plants have been being used as remedy for disease for a long time. They not only cure the disease but also provide an important role in the economy. Medicinal plants are cheap and easy to get to those people who knew it very well. Bioactive compounds are deposited in medicinal plants; it can serve as important raw materials for pharmaceutical manufacturing. They comprise a precious asset of a country and donate to its health care system. Well-judged and scientific investigation of this wealth can significantly contribute to the public health. Besides being available commodity of commerce. A country can also earn a good amount of foreign currency by exporting this natural wealth to other countries. More than 500 of such medicinal plants have so far been established as growing in Bangladesh[20].Almost all of these indigenous medicinal plants are extensively used in the preparation of unani, ayurvedic and homeopathic medicines in Bangladesh.

A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% of people suffering from illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mullahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in crude and substandard form, by our people[21].Traditional medicines are still manufactured in our country by following the age-old unscientific, traditional methods. Hundreds of indigenous medicinal plants are employed in different Ayurvedic and Unani commercial preparations without proper standardization, quality control, evaluation and determination of the chemical nature, pharmacological and toxicological studies of the active components which are essential to utilize their therapeutic potential fully. Toxicity of the plants or plant extracts is coming to light with the advancement of science. 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 possible only by developing standard drugs from our natural resources of medicinal plants. In order to achieve this goal research and development of traditional medicines should be given the due priority[20].Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to manufacture drugs. Each year a great deal of money is spent on this purpose.

1.4 General Approaches to Drug Discovery from Natural Sources

In general, three different approaches have been, and continue to be used in the drug discovery process from natural sources. These approaches are: traditional empirical and molecular[22].During the Vedic period the ‘Susrutasamhita’ and the “charakasamhita” were influential works on traditional medicine. Hundreds ofmedicinal plant were identified and have been traditionally used since then. Over the following centuries, Ayurvedic practitioners developed a number of medicinal preparations and surgical procedures for the treatment of various ailments and diseases. WHO (World Health organization) estimates that 80% of the populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the traditional plants play a major role and constitute the backbone of the traditional medicine. Indian Materia medical includes about 1600 drugs of vegetable origin almost all of which are derived from different traditional system and folklore practices[23].Examples include drugs like morphine, quinine and ephedrine that have been in widespread use for a long time, and more recently adopted compounds such as the antimalarial artemisinin. The empirical approach builds on an understanding of a relevant physiological process and often develops a therapeutic agent from a naturally occurring lead molecule. Examples include tubocurarine and other muscle relaxants, propranolol and other β-adrenoceptor antagonists, and cimetidine and other H_2

receptor blockers. Development of molecular biological techniques and the advances in genomics lead to molecular approach. The molecular approach to drug discovery can be further subdivided into three general categories. The first is rational drug design using computer aided techniques. A second area is the antisense approach, which is based on manipulation of genetic targets. The third technique, which currently dominates drug discovery activity, is the pragmatic approach of random screening. With recent technological developments in molecular biology, instrumentation and information technology, screening of compounds can be conducted by high throughput screening method. High throughput screening is an automated testing process of large number of compounds versus a large number of targets which is particularly effective in identifying potential lead compounds. Robotics and miniaturization of in vitro tests on genetically modified cells has lead to high throughput screening[22].The major advantage of natural products for random screening is the structural diversity. Since Bioactive natural products often occur as a part of a family of related molecules, it is therefore possible to isolate a number of homologues compounds and obtain structure-activity relationship. Of course, lead compounds found from screening of natural products can be optimised by traditional medicinal chemistry or by application of combinatorial approaches. Overall, when faced with molecular targets in screening assays for which there is no information about low molecular weight leads, use of a natural products library seems more likely to provide the chemical diversity to yield a hit than a library of similar numbers of compounds made by combinatorial synthesis. Since only a small fraction of the world's biodiversity has been tested for biological activity, it can be assumed that natural products will continue to offer novel leads for novel therapeutic agents[22].In earlier times, all drugs and medicinal agents were derived from natural substances, and most of these remedies were obtained from higher plants. Today, many new chemotherapeutic agents are synthetically derived, based on "rational" drug design. The study of natural products has advantages o'er synthetic drug design in that it leads optimally to materials having new structural features with novel biological activity. Not only do plants continue to serve as important sources of new drugs, but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivity. The starting materials for about one-half of the medicines we use today come from natural sources. Virtually every pharmacological class of drugs includes a natural product prototype. The future of plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising[24].

1.5 Phytotherapy

The treatment of diseases by the use of the plants is known as phytotherapy. Phytotherapy was the beginning of pharmacotherapy or treatment of disease by means of drugs. Therapeutics uses of plants had in effect stored at the very beginning of human life on the earth when the primitive man out of necessity and by intuition, restored to using plants to alleviate suffering from injuries and diseases[25].Phytotherapy laid the foundation stone of all from of medical treatment that are practiced today. With the development of human civilization of phytotherapy exhibits a stepwise development, which can be enumerated as-

1st stage: Crude drugs were employed, prepared in the roughest manner, such as powdered willow in the management of pain.

2nd stage: These were converted into more active and manageable forms, such as extracts or solutions watery or alcoholic.

3rd stage: The pure active principles separated from crude drug were employed e.g. salicylic acid.

4th stage: Attempt to synthesize the active drug in the laboratory and indeed structural modification. e.g. Aspirin.

1.6 Medicinal plants and drug development

Development of drugs from medicinal plants is often an elaborate laborious time consuming and expensive exercise. Careful phytochemical analysis and pharmacological and clinical tests are pre-requisites for developing drugs from medicinal plants. The stage involve in the following way development exercise may be summarize as follows:

(i) Selection and correct identification of the proper medicinal plants and its extraction with a suitable solvent.

(ii) Detection of biological activity of the crude extent and establishment of a bioassay system to permit the identification of the active fractions and rapid discarding of the inactive ones.

(iii) Fraction of the crude extracts by using physico-chemical procedure and monitored by biological tests identification and separation of the active fractions.

(iv) Isolation of the active constituents by chromatographic or other technique and purification of the isolation compounds by repeated chromatography and crystallization.

(v) Establishment of the chemical structures of the pure compound by various physicochemical techniques and determination of their biological activity by various pharmacological tests.

1.7 Contribution of medicinal plants to modern drug

Plants have contributed and are still contributing to the development of modern synthetic drugs and medicine in a number of ways as stated below:

(i) Novel structure of biologically active chemical compounds isolated from plants sources, often to prompt the chemists to synthesis similar or better semi —synthetic compounds.

(ii) Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant derived compound with known biological activity.

(iii) Chemists for use as potent drugs often prepare various analogues of derivatives of plant constituents with similar or better pharmacological action and therapeutics properties.

Homatropine (a synthetic tropane alkaloid similar to atropine) syrosingopine (a synthetic derivative of reserpine) chloroquine (a synthetic derivative of quinine) dihydromorphinone, oxymorphine, methyldihydromorphinone, ethyl morphine and N-allylnormorphine (synthetic derivatives of morphine) are some of the example of such synthetic drug which plants have contributed indirectly. Even in this age of synthetic drug there are some naturally occurring drugs, such as the Digitalis glycoside used in cardiac complications and the Catharanthus alkaloids used in cancers, which have no synthetic alternatives. In such cases plants continue to remain as their principal and oily sources.

1.8 Status of medicinal plants of Bangladesh

(i) About 500 medicinal plants have been reported to occur in Bangladesh

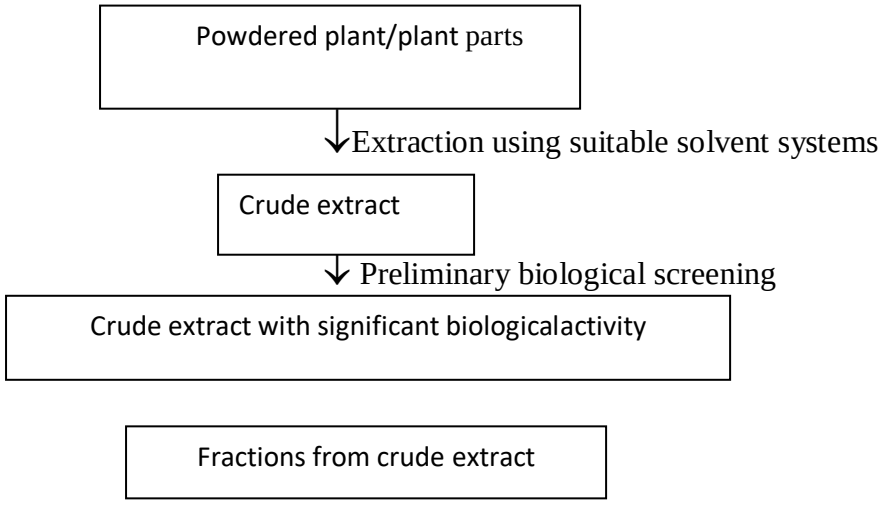
(ii) Almost 80% of rural populations are dependent on medicinal plants for their primary health care.
(iii) The local people conserve traditional knowledge through their experience and practices which is handed down orally without any documentation.
(iv) The over exploitation of wild medicinal plants has become a threat to its extinction.
(v) In Bangladesh there is no systematic cultivation process of conservation strategies about medicinal plants.
(vi) There is no government policy or rules and regulations about the medicinal plants cultivation conservation and marketing.
(vii) There are almost 422 herbal medicinal companies using medicinal plants as raw materials mostly by importing from abroad.

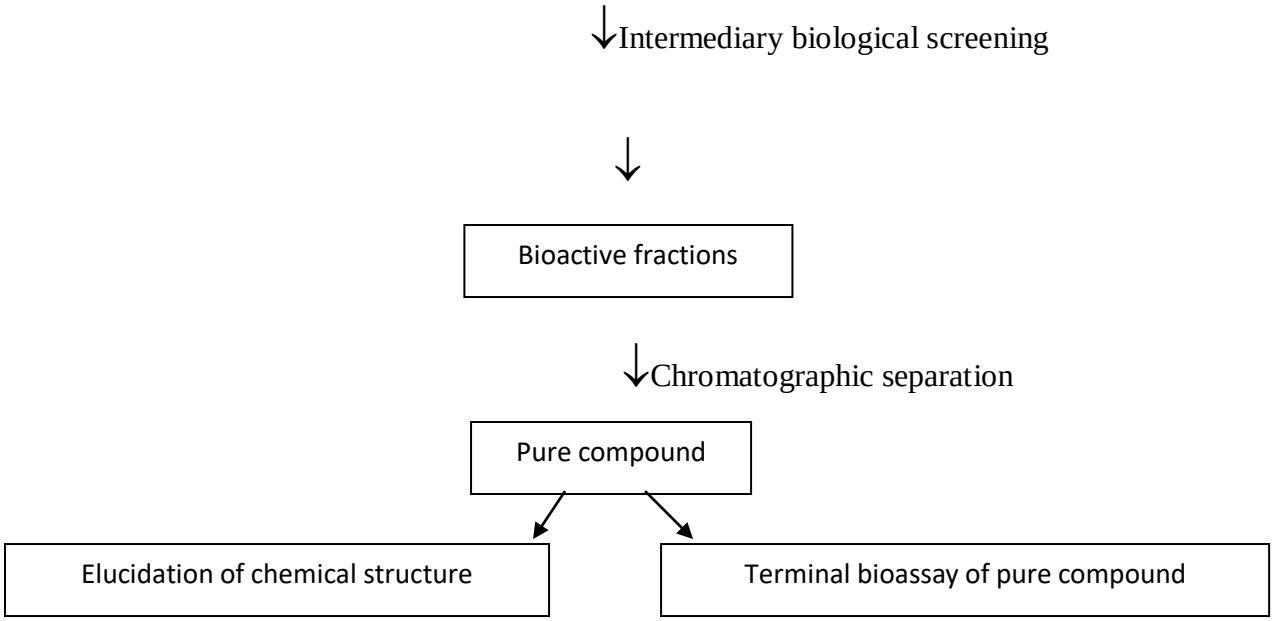
1.9 Chemical constituents of medicinal plants

The commonly occurring chemical substances which are responsible for the medicinal (as well as toxic) properties of plants include the following-

1. Volatile or essential oils
2. Fixed oils
3. Gum-resins and mucilage
4. Alkaloid and amines
    a. Pyridine group
    b. Tropane group
    c. Isoquiloline group
    d. Quinoline group
    e. Quinolizidile group
    f. Indole group
    g. Steroidal group
    h. Phenylethylamine group
    i. Alkaloid amines
5. Glycosides:
    a. Anthraquinone glycoside
    b. Cardiac glycoside
    c. Saponin glycoside Thiocyanate glycoside
    d. Other glycoside
6. Vitamin and mineral

1.10 Flow Chart of Bioactivity Guided Phytochemical Approach





The objective of this dissertation is to identify the biological activity of the roots of an indigenous medicinal plant, viz., *Ficusracemosa* (Family: Moraceae) and to evaluate the possible phytochemical and pharmacological profiles of the crude extracts. So far some chemical and biological investigations have been carried out on this plant mainly focusing on the bark and root of the plant. That's why the goal of this framework is to explore the potential possibilities of developing new drug candidates from the fruit of this plant which could be crucial for the treatment of various ailments.

Planet preview:

2 .1: The plant family: Moraceae

Ficusracemosa is an attractive fig tree with a crooked trumk and a spreading crown. Unlike the banyan, it has no aerial roots. The most distinctive aspect of this tree is the red, furry figs in short clusters, which grow directly out of the trunk of the tree[26].

Leaves: The leaves are dark green, 6-10 cm long, glabrous; receptacles small subglobose or piriform, in large clusters from old nodes of main trunk.

Fruits: The fruits receptacles are 3-6 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 *FicusRacemosa* Linn is 3/4inch to 2 inches long, circular and grows directly onthe trunk[27].

Flowers: Looking for the flower of *Ficusracemosa* should know that the fig is actually a compartment carrying hundreds of flowers. One might wonder how these flowers enclosed in a ball are pollinated. The flowers are pollinated by very small wasps that crawl through the opening in search of a suitable place to reproduce (lay eggs). Without this pollinator service fig trees cannot reproduce by seed. In turn, the flowers provide a safe haven and nourishment for the next generation of wasps.

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.

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[28].

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.

2.1.1: Vernacular names

Scientific Name: *Ficusracemosa*
Synonyms: *Ficusracemosa* var. *mollis, Ficusglomerata*
Bengali: Dumor, উদুম্বর,Udumbara
English: Gular fig, Country Fig, Cluster Fig
Gujarati: Goolar, Umbaro
Hindi: Dumar, Jantu Phal, Goolar, Goolar, Pani Bhuj, Pushp-hina, Udumbara, Dharma Patra, Umari
Malayalam: Aththi, Udumbaram, Atthi, Jantuphalam, Atthi-al
Other name: Dumrii, Redwood Fig, Indian Fig, Cluster Fig, Atteeka, Goolar, Vellaiatthi, Crattock, Country Fig, Gular Fig, Rumbodo, Athi
Oriya: Dimri
Tamil: Anai, NallaAtthi, MalaiyinMunivan, VellaiAtthi, Atti, Utumparam, Atti
Telugu: Atti, Bodda, Brahmamamidi
Urdu: Dumar

2.1.2: Recent taxonomic revisions of the family include these genera: [29][30].

- *Ficuschittagonga*
- *Ficusglomerata*
- *Ficusglomerata* var. *chittagonga*
- *Ficusglomerata* var. *elongata*
- *Ficusglomerata* var. *miquelii*
- *Ficusglomerata* var. *mollis*
- *Ficusgoolereea*
- *Ficushenrici*
- *Ficuslanceolata*
- *Ficuslucescens*
- *Ficusmollis*
- *Ficusracemosa* var. *miquelii*
- *Ficusracemosa* var. *mollis*
- *Ficusracemosa* var. *vesca (F. Müll. ex Miq.) Barrett*
- *Ficussemicostata*
- *Ficustrichocarpa*

2.2: Classification (*Ficusracemosa* L. Taxonomic Serial No.: 506544)

Domain: Eukaryota
Kingdom: Plantae
Sub-kingdom: Viridiplantae
Phylum: Magnoliophyta
Sub-phylum: Euphyllophytina
Infra-phylum: Radiatopses
Division: Tracheophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Rosales

Family: Moraceae
Genus: *Ficus*
Species: *Ficusracemosa*

Figure 2.1: *Ficus racemosa* plant (source: Tropical Ferns).

Figure 2.2: The flowers of *Ficusracemosa* (source: Ayushvedah).

Figure 2.3: Leaves of *Ficusracemosa* (Source: Alchetron)

2.3 Plant Description

Ficusracemosa, 20 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[31].Leaves simple, alternate, stipules 12-18 mm long, lanceolate, linearlanceolate, 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 oblongovate, 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, ovatetriangular, 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[32].Syconium 2.5 x 2 cm, orange, pink or dark crimson; achene granulate.

Scientific name: *Ficus racemosa*.
Common name: Dumor.
Family: Moraceae
Plant type: Evergreen and Shrub
Origin: The plant is found in Bangladesh, India, Pakistan, China, Myanmar, Thailand, Indonesia, Vietnam, Australia etc**.**
Ecology: *Ficusracemosa* a well-known medicinal plant commonly known as Dumor in Bengali is indigenous to Bangladesh. The farmers on the roadside and fallen land can also cultivate *Ficusracemosa.* This bush grows in all parts of the worlds. This plant occurs throughout Bangladesh. This shrub grows on the plains of Bangladesh and in the lower regions, up to a range of 1000 meters above sea level. This plant is also cultivated in other

tropical areas. It is also distributed in India, Myanmar, Thailand Indonesia Vietnam, Australia etc.

Cultivation: Ficus species are common and form an important element of lowland rain forest, both as canopy and understorey trees. Most species prefer per-humid forest, but several are found in areas with a monsoon climate and in teak forest, including locations where the soil dries out. Succeeds in full sun to partial shade. Succeeds in most soils that are reasonably moist but well-drained. Cluster fig is resistant to fire.

Parts used: Root, leaf, flower, fruit are used for therapeutic use. The leaves, roots, flowers and stem bark of this plant are used in medicinal applications.

2.5 Chemistry of the plant *Ficusracemosa*

Racemosa Linnspecies contain flavanoid glycosides , alkaloids , phenolic acids , steroids , saponins , coumarins , tannins , triterpinoids – oleanolic acid rusolic acid , α- hydroxy ursolic acid , protocatechuic acid , maslinic acid . The nonenzymatic constituents include phenolic compounds, flavonoids, vitamin C. The enzymatic constituents present are ascorbate oxidase, ascorbate peroxidise, catalase, peroxidise. The phenolic compounds present are gallic acid and ellagic acid[33].

2.6 Medicinal uses of the plant *Ficusracemosa*

- The leaves are used in the treatment of diarrhea.
- The bark is astringent. It is used in the treatment of haematuria, menorrhagia, and haemoptysis.
- The fruit is astringent. It is used in the treatment of haematuria, menorrhagia, and haemoptysis. The fruit, when filled with sugar, is considered to be very cooling.
- A fluid that exudes from the cut roots of the tree is considered to be a powerful tonic when drunk for several days together. The sap is a popular remedy in Bombay, that is applied locally to mumps and other inflammatory glandular enlargements, and is also used in the treatment of gonorrhoea.
- The root is chewed as a treatment for tonsilitis.

2.6.1: Medicinal Applications of the plant *Ficusracemosa*

A few of the health benefits derived from figs include-

Prevention of constipation: There are 5 grams of fiber in every three-fig serving. That high concentration of fiber helps promote healthy, regular bowel function and prevents constipation. Fiber works to add bulk and mass to bowel movements, so it not only prevents constipation, but also eliminates diarrhea and unhealthy or irregular bowel movements.

Weight loss: The fiber in *Ficusracemosa* also helps to reduce weight and is often recommended for obese people. However, their high calorie count can also result in weight gain, especially when consumed with milk. A few figs are enough to get the recommended amount of nutrients, so don't overdo it! Remember, it is possible to have too much of a good thing.

Lower cholesterol: *Ficusracemosa* contain Pectin, which is a soluble fiber. When fiber moves through the digestive system, it basically mops up excess clumps of cholesterol and carries them to the excretory system to be eliminated from the body. As a soluble fiber, pectin from figs also stimulates healthy bowel movements. Figs can have a laxative effect, as they are one of the most fiber-dense foods available. High amounts of fiber in your diet can benefit your overall health by preventing certain types of abdominal cancer, as well as colon cancer.

Prevention of coronary heart disease: Dried figs contain phenol, Omega-3 and Omega-6. These fatty acids reduce the risk of coronary heart disease. Furthermore, the leaves of figs have a significant effect on the level of triglycerides in a person's system. Fig leaves have an

inhibitory effect on triglycerides, and makes the overall number of triglycerides drop. Triglycerides are another major factor behind various heart diseases.

Prevention of colon cancer: The presence of fiber helps to stimulate the elimination of free radicals and other cancer causing substances, particularly in the colon, since fiber increases the healthy movement of the bowels.

Protection against post-menopausal breast cancer: Fiber content in figs have been known to protect against breast cancer, and after menopause, the hormonal balance in women can often fluctuate. The body's systems are so interconnected that hormones affect the immune system, which is turn affect the ability of antioxidants to fight free radicals. Free radicals are prime factors behind the development of cancer, so figs take care of one extra line of defense by providing its wealth of fiber.

Good for diabetic patients: The American Diabetes Association recommends figs as a high fiber treat that helps promote functional control of diabetes. Fig leaves reduce the amount of insulin needed by diabetic patients who have to regularly take insulin injections. *Ficusracemosa* are rich in Potassium, which helps to regulate the amount of sugar which is absorbed into the body after meals. Large amounts of potassium can ensure that blood sugar spikes and falls are much less frequent, so figs can help diabetics live a much more normal life.

Prevention of hypertension: People usually take in sodium in the form of salt, but low potassium and high sodium level may lead to hypertension. Figs are high in potassium and low in sodium, so they are a perfect defense against the appearance and effects of hypertension, making figs a relaxing food as well, which can settle the nerves and bring some calmness to your day.

Bronchitis: The natural chemicals in *Ficusracemosa* leaves make it an ideal component for a tea base. Fig leaf tea has been popularly prescribed for various respiratory conditions like bronchitis, and it is also used as a way to prevent and lessen the symptoms of asthmatic patients.

Venereal Diseases: Figs have been traditionally used in the Indian subcontinent and a few other areas of the world as a calming salve for venereal diseases. Ingestion or topical application both work for relief from sexually transmitted diseases, although further research needs to be done on the exact range of symptoms and diseases which figs positively effect.

Sexual Dysfunction: For centuries, *Ficusracemosa* have been recommended as a way to correct sexual dysfunction like sterility, endurance, or erectile dysfunction. It has been a major part of mythology and culture, and most of the time, it is referenced as a powerful fertility or sexual supplement. Its actual success as an aphrodisiac is questionable, but the huge amount of valuable vitamins and minerals might result in the sudden boost in energy and stamina that people mistake for a sexual surge. Soak 2-3 figs in milk overnight and eat them in the morning to enhance your sexual abilities.

Strengthens Bones: *Ficusracemosa* are rich in calcium, which is one of the most important components in strengthening bones, and reducing the risk of osteoporosis. It is also rich in phosphorus, which encourages bone formation and spurs regrowth if there is any damage or degradation to bones.

Urinary calcium loss: People that maintain a high-sodium diet may be affected by increased urinary calcium loss. The high potassium content in figs helps to avoid that condition and regulates the content of waste in urine and minimizes the calcium lose, while increasing the amount of uric acid and other harmful toxins which you want to get out of your body.

Prevention of macular degeneration: Vision loss in older people is normally due to macular degeneration. Fruits and figs are particularly good at helping you avoid this very common symptom of aging.

2.6.2: Pharmacological Activities of *Ficusracemosa*

Antimicrobial activity

Methanolic extracts of *Ficusracemosa* showed significant activity against four clinical strains of bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, and *E. coli*. The standard drug used was tetracycline (100 mcg/ml). In both the cases, the methanolic extracts at dose concentration of 500μg/ml showed significant antimicrobial activity.

Analgesic activity

Saponin (SN) isolated from *Ficusracemosa* flowers exhibited protection from writhing induced by 1.2% v/v acetic acid in Adult Swiss albino mice. SN1 was administered ip at doses of 30, 50, 75 and 100 mg/kg and standard drug used were aspirin, paracetamol and morphine sulphate. In hot plate method, SN not only produced analgesia in mice but also potentiated the analgesic action of pentazocine and aspirin[34].

Antibacterial activity

The methanol extract of flowers of the plant have been screened for their antibacterial activity. The extract (7.5 mg/disc) showed broad-spectrum antibacterial activity against gram positive and gram negative bacteria. The results were compared with the standard drug streptomycin (10 µg/ disc). The zone inhibition was found to be increased with the increase in concentration of the extract and thus exhibiting concentration dependent activity[35].

Preparetion of planet extracts:

3.1 Biological investigation of the experimental plants

A plant species representing to the family: *Ficusracemosa* was investigated in this study.

Name of plant	Family	Plant part
Ficusracemosa	Moraceae	Flower

The investigations of the plant will be discussed in two different sections.

- Phytochemical Investigation, and
- Biological Investigation.

3.2 Biological investigation of *Ficusracemosa*

3.2.1 Collection and preparation of the plant material

The whole plant of *Ficusracemosa*was collected from Khamarbari, Farmgate, Dhaka and identified by taxonomist of National Herbarium, Bangladesh situated at Mirpur in Dhaka. The sample is preserved in the Phytochemical Laboratory of World University of Bangladesh for as further reference (Accession No. 41878). Fruits air dried for several days and then oven dried for 24 hours at considerably low temperature (not more than 40^0C) for better grinding. The dried fruits were then ground to a coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, World University of Bangladesh (WUB).

3.2.2 Extraction of the plant material

The powdered material (250 gm) was taken in a cleaned, amber colored reagent bottle (5 liters) and soaked in 1.5 L of methanol. The container with its content was sealed by bottle cap and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then allowed to evaporate at ambient temperature until approximately 70% solvent was evaporated.

3.2.3 Solvent-Solvent partition of crude extract by Modified Kupchan Partition method

Solvent-solvent partitioning was done using the protocol designed by Kupchan[36].The crude extract (5 gm) was dissolved in 10% aqueous methanol. It was extracted with Petroleum ether, then with carbon tetrachloride and finally with Chloroform. The whole partitioning process is schematically shown in Figure 3.1. All the five fractions were evaporated to dryness (Table 3.1) and were used for further analysis.

3.2.3.1 Partitioning with Petroleum ether

The mother solution was taken in a separating funnel. 100 ml of the Petroleum ether was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice and the fractions collected were evaporated together in Rotary evaporator.

3.2.3.2 Partitioning with Carbon Tetrachloride

To the mother solution left after partitioning with petroleum ether, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with carbon tetrachloride (100 ml x3). The carbon tetrachloride fractions were collected together and evaporated. The aqueous fraction was preserved for the next step.

3.2.3.3 Partitioning with Chloroform

To the mother solution that left after washing with petroleum ether and carbon tetrachloride, 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with Chloroform ($CHCl_3$) (100 ml X 3). The $CHCl_3$ soluble fractions were collected together and evaporated. The aqueous methanol fraction was preserved as aqueous fraction.

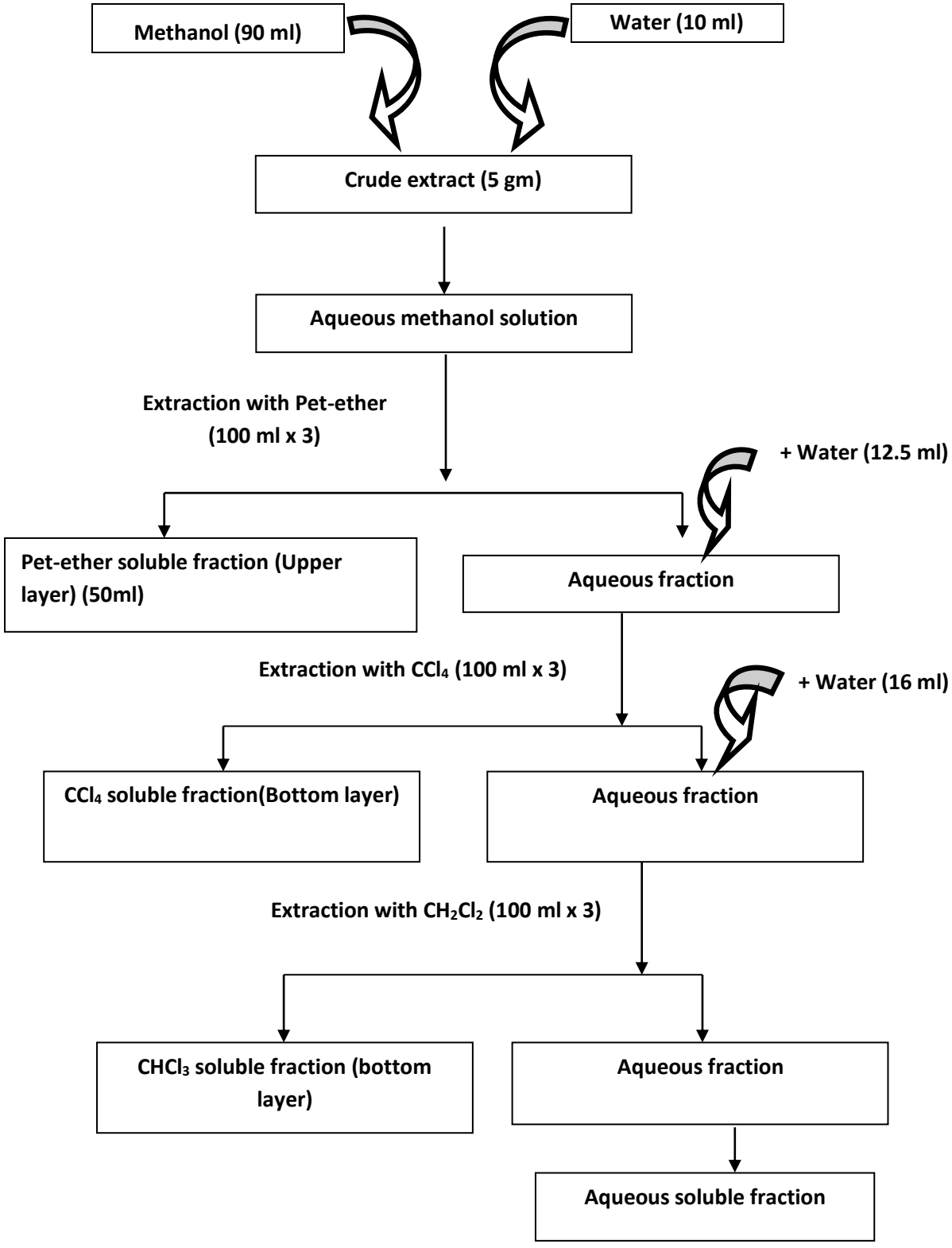


Figure 3.1: Schematic representation of the modified Kupchan Partitioning of crude extract of root of *Ficusracemosa*

P

Petroleum Ether Soluble Fraction
Figure 3.1: Partitioning of plant extract

Table 3.2: Fractions obtained from *Ficusracemosa* extract (Author's own work).

Plant part	Sample code	Fraction
Flowers of *Ficusracemosa*	**MESF**	Methanol extract
	PESF	Pet-ether soluble fraction
	CTCSF	Carbon tetrachloride soluble fraction
	CSF	Chloroform soluble fraction
	AQSF	Aqueous Soluble Fraction

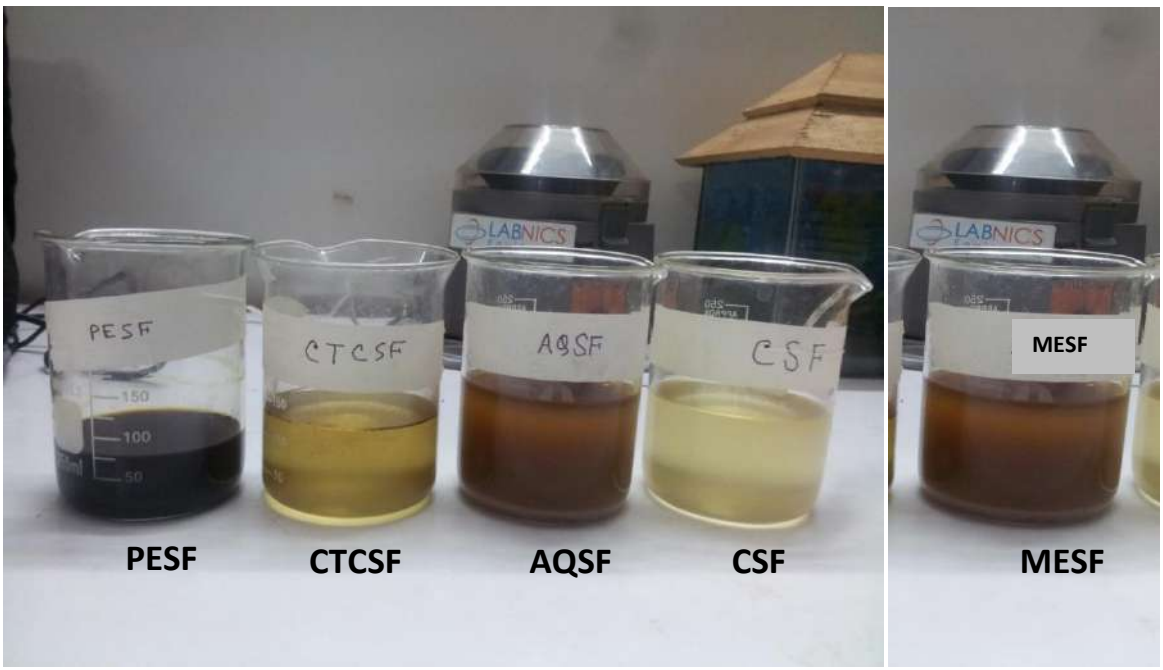


Figure 3.3: Different fractions of *Ficus racemosa* (Author's own work).

Design of biological Investigation:

4.1 General Approaches to Drug Discovery from Natural Sources

Natural products have been the most successful source of drugs ever. Research progressed along two major lines: ethno pharmacology (medicinal herbs, substances of abuse, ordeal poisons) and toxicology (poisonous plants, venomous animals, arrow and fish poisons). These strategies have already produced many valuable drugs and are likely to continue to produce lead compounds. Approximately 60% of the world's population relies entirely on plants for medication[37].Of the 520 new drugs approved between 1983 and 1994, 39% were natural products or derived from natural products and 60–80% of antibacterial and anticancer drugs were derived from natural products. Thirteen natural product related drugs were approved from 2005 to 2007, and five of these represented the first members of new classes of drugs: the peptides exenatide and ziconotide, and the small molecules ixabepilone, retapamulin. Current commercial evidence also supports the case for natural products. Of the 20 bestselling non-protein drugs in 1999, nine were either derived from or developed as the result of leads generated by natural products simvastatin, lovastatin, enalapril, pravastatin, atorvastatin, augmentin, ciprofloxacin, clarithromycin and cyclosporin with combined annual sales of >US$16 billion. Newer developments based on natural products include the antimalarial drug artemisinin and the anticancer agent's taxol, docetaxel and camptothecin[38].

Today, many new chemotherapeutic agents are synthetically derived, based on "rational" drug design. The study of natural products has advantages over synthetic drug design in that it leads optimally to materials having new structural features with novel biological activity. Not only do plants continue to serve as important sources of new drugs, but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivity.

Natural products are naturally derived metabolites and/or by products from microorganisms, plants, or animals. The major advantage of natural products for random screening is the structural diversity. Bioactive natural products often occur as a part of a family of related molecules so that it is possible to isolate a number of homologues and obtain structure-activity relationship. Of course, lead compounds found from screening of natural products can be optimized by traditional medicinal chemistry or by application of combinatorial approaches. Overall, when faced with molecular targets in screening assays for which there is no information about low molecular weight leads, use of a natural products library seems more likely to provide the chemical diversity to yield a hit than a library of similar numbers of compounds made by combinatorial synthesis. Since only a small fraction of the world's biodiversity has been tested for biological activity, it can be assumed that natural products will continue to offer novel leads for novel therapeutic agents.

4.2 Investigations

The crude extract was subjected for chemical group tests and identified for - steroids, alkaloids, tannins, gums, flavonoids, reducing sugar and saponins. Results of different group tests are given in table 4.1.

Table 4.1 Results of different chemical group test of the Methanol extract of *Ficusracemosa*

Sample	Test solution	Observation	Inference
Tests for Alkaloids: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid	1 ml of Mayer's reagent.	Yellowish colored precipitate was obtained.	Presence of alkaloid.
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid.	1 ml of Dragendroff's reagent.	Orange brown precipitate was observed.	Presence of alkaloid.
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid	1 ml of Hanger's reagent.	Yellowish precipitate was obtained.	Presence of alkaloid.
Test for Steroids: 1 ml solution of chloroform extract	1 ml of chloroform & few drops of con. Sulfuric acid	Red color was observed.	Presence of steroid.
Test for Flavonoids: 1 ml solution of ethanol extract.	Few drops of lead acetate was added to the extract.	Yellow color was formed.	Presence of Flavonoids.
Test for Saponins: 1 ml solution of the extract was diluted with distilled water to 20 ml.	Shaken in a graduated cylinder for 15 minutes	One-centimeter layer of foam was formed.	Presence of saponins.
Tests for Tannins: 5 ml solution of extract.	1 ml of 5% Ferric chloride solution.	Greenish black precipitate was formed.	Presence of tannins.
5 ml solution of extract.	1 ml of 10% potassium dichromate solution.	Yellow precipitate was obtained.	Presence of tannins.
5 ml solution of extract.	1 ml of 10% led acetate solution.	Yellow precipitate	Presence of tannins.
Test for Reducing Sugars: 2 ml solution of aqueous extract.	2 ml equal volume of Fehling's A and B solution. Boiled for 5 minutes on a boiling water bath.	Brick red colored precipitate was found.	presen of reducing sugars.
Test for Gums : 5 ml solution of extract.	1ml molisch reagent and 1ml con. Sulfuric acid were added.	Red violet ring was produced at the junction of two liquids.	Presence of gums

4.3 Results & Discussions

Phytochemical studies showed that Alkaloids, Steroids, Flavonoids, Tannins, Saponin, Gum & Reducing sugars are present in the methanol extract of the plant. The experimental findings from the study showed that the methanol extract has some important groups and secondary metabolites that can show extensively pharmacological activity.

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world due to their-

4.3.1 Microbiological investigations

The antibacterial as well as antifungal spectrum of the crude extracts can be ascertained by observing the growth response with the help of in vitro antimicrobial study. These experiments are rationalized on the fact that many infectious diseases are caused by bacteria and fungi and if the test materials inhibit bacterial or fungal growth then they may be used in those particular diseases.

However, a number of factors can influence the results like-

- The extraction method
- Inocula volume,
- Culture medium composition,
- pH and
- Incubation temperature

4.3.2 Thrombolytic activity investigation

Cerebral venous sinus thrombosis (CVST) is a common disorder that is often accompanied by significant morbidity and mortality. In anticoagulation therapy the intravenous heparin is the first line of treatment for CVST, because of its efficacy, safety and feasibility. However, thrombolytic therapy with its ability to produce rapid clot lysis has long been considered as an attractive alternative. Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of patients with CVST.

4.3.3 Membrane stabilizing activity investigation

Inflammatory cells produce a complex mixture of growth differentiation cytokines as well as physiologically active arachidonate metabolites .In addition they possess the ability to generate reactive oxygen species (ROS) that can damage cellular biomolecules which in return augments the state of inflammation[39].The erythrocyte membrane resembles to lysosomal membrane and such as the effect of drugs on the stabilization of erythrocytes can be extrapolated to the stabilization of lysosomal membrane[40].Therefore when membrane stabilizes they interfere in the release and in the action of mediators like histamine, serotonin, prostaglandine, leukotrines etc[41].

4.3.4 Analgesic activity investigation

Due to having adverse side effects like gastric lesion caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. So, analgesic drugs are being searched all over the world as alternatives to NSAIDs and opiates. The investigation of the efficacy of plant based drugs used as traditional medicines have been paid great attention because they are-

- Cheap
- Have little side effects

According to WHO, about 80% of the world's population rely mainly on plant based drugs? So in these dissertation two types of analgesic activity has been evaluated-

- Peripheral analgesic activity
- Central analgesic activity

4.3.4 Hypoglycemic activity investigation

The origin of the term is Greek: “hyper”, meaning excessive; “glyc”, meaning sweet; and “emia”, meaning of the blood Hyperglycemia, or high blood sugar, is a condition in which an excessive amount of glucose circulates in the blood plasma. This is generally a glucose level higher than (120 mg/dl) but symptoms may not start to become noticeable until even higher values such as 250–300 mg/dl. A subject with a consistent range above 126 mg/dl is generally held to have hyperglycemia. Chronic levels exceeding 125 mg/dl can produce organ damage[42].Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for improved and safe natural anti-diabetic agents is ongoing. The plant kingdom offers a wide field to look for oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only few of them have been investigated[43].and the World Health Organization has recommended that this area warrants attention[44]. This study was thus undertaken to evaluate the hypoglycemic effect of a methanolic extracts of *Ficusracemosa* flowers and their different fractionates.

4.3.5 Anti-diarrheal activity investigation

Diarrhea is characterized by an increase in the frequency of the bowel movements, wet stool and abdominal pain. It is the world’s third highest killer disease, contributing substantially to pediatric morbidity and mortality, especially in the malnourished. The incidence of diarrhea is still high (about 7.1 million per year), despite the effort of the international organization to control the disease. Thus in this study, the anti-diarrheal activity was investigated by giving the plant extractives. Here, the plant extractive causes the inhibition of excessive peristaltic movement induced by oral administration of castor oil.

Evaluation of Total Phenolic content (TPC):

5.1: Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which are based on their uses in traditional medicine. Plants produce a diverse array of bioactive molecules that are particularly important in the treatment of life-threatening conditions[45].Oxidation reactions initiated by excess free radicals have been shown to lead to the formation of tumors, damage of DNA, mRNA, proteins, enzymes; cause cancer, cardiovascular diseases, nervous disorders, premature ageing, Parkinson's and Alzheimer's diseases, rheumatic and pulmonary disorders[46].Therefore, the need for systematic screening of medicinal plants for antioxidant activity cannot be overemphasized.

Free radicals are atoms or group of atoms that have at least one unpaired electron, making them highly reactive. The potentially reactive derivatives of oxygen are known as reactive oxygen species (ROS) (e.g. superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals), and play an important role in oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA, related to the pathogenesis of various important diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing process.

Antioxidants are the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance. Antioxidants prevent the oxidative damage by directly reacting with ROS, quenching them and/or chelating catalytic metal ions and also by scavenging free oxygen. Since ancient times, many herbs have been potentially used as an alternative remedies for treatment of many infections, diseases and as food preservatives suggesting the presence of antimicrobial and antioxidant constituents[47].There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases[48].Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc., are some of the antioxidants produced by the plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used antioxidants.

Different synthetic antioxidant such as *tert*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) used as food additives to increase shelf life are known to have not only toxic and carcinogenic effects in humans, but abnormal effects on enzyme systems. Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years. Plant polyphenols have been studied largely becauseof the possibility that they might underlie the protective effects afforded by fruit and vegetable intake against cancer and otherchronic diseases.

Because of the complex nature of phytochemicals, the antioxidant activities of plant extracts must be evaluated by combining two or more different *In-vitro*assays. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade.

The purpose of this study was to evaluate different extractives of roots of *Ficusracemosa* as new potential sources of natural antioxidants and phenolic compounds.

5.2: Assays for total phenolic content

The anti-oxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The phenolic compounds exert their antioxidant

properties by redox reaction, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations. Phenolic compounds are secondary metabolites of plants and can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelating of metal ions[49].

5.2.1: Principle

In the alkaline condition phenols ionize completely. 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 becomes blue. The intensity of the color change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound [50].

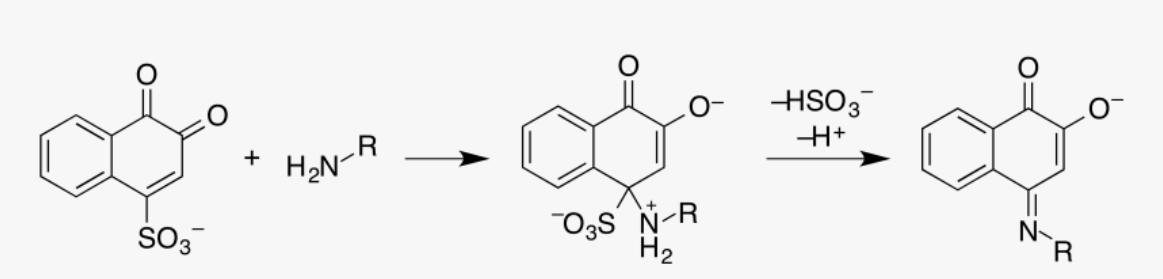


Figure 5.1. Folin Ciocalteu Reagent Reaction with Phenol (Source: PNGitem)

5.2.2: Materials & Methods

Total phenolic content of root of Phytochemical and biological investigations of *Ficusracemosa* extractives was measured employing the method as described by[51]. involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard[52].

5.2.2.1: Materials

- Folin-Ciocalteu reagent (10 fol diluted)
- Na_2CO_3 solution (7.5 %)
- tert-butyl-1-hydroxytoluene (BHT)
- Ascorbic acid
- Methanol
- Chloroform
- Carbon tetra chloride
- Pet-ether
- Distilled water
- UV-spectrophotometer
- Vial
- Beaker (100 & 200ml)
- Test tube
- Pipette (1ml)
- Pipette (5ml)
- Micropipette (50-200 µl)

5.2.2.2: Composition of Folin-Ciocalteu reagent [53].

SL.No.	Component	Percent
1	Water	57.5
2	Lithium Sulfate	15.0
3	Sodium Tungstate Dihydrate	10.0
4	Hydrochloric Acid≥25%	10.0
5	Phosphoric Acid 85 % solution in water	5.0
6	Molybdic Acid Sodium Dihydrate	2.5

5.2.3: Standard curve preparation

Gallic acid was used here as standard. Different gallic acid solutions were prepared having a concentration ranging from 100 µg / ml to 0 µg / ml. 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 0.5 ml of gallic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

5.2.4: Sample preparation

2 mg of the extractives was taken and dissolved in the distilled water to get a sample concentration of 2 mg / ml in every case. The samples along with their concentration for the total phenolic content measurement are given in the Table 5.1.

Table-5.1: Test sample for total phenolic content determination

Plant part	Sample code	Test Sample	Calculated amount (mg/ml)
Flowers of *Ficusracemosa*	MESF	Methanol extract of fruits of *Ficusracemosa*	2.0
	PESF	Pet-ether soluble fraction	2.0
	CTCSF	Carbon tetrachloride soluble fraction	2.0
	AQSF	Aqueous soluble fraction	2.0

5.2.5: Total phenolic content analysis

To 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration, the total phenolic content of the samples was measured. The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract [54].

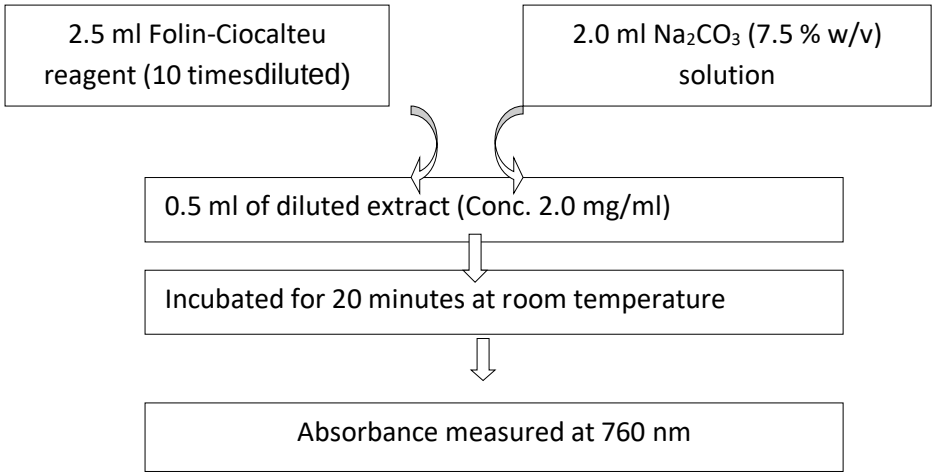


Figure: 5.2: Schematic representation of the total phenolic content determination.

5.3: Results and discussion of the test samples of fruits of *Ficusracemosa*

5.3.1: Total phenolic content (TPC)

The methanolic extract of *Ficusracemosa* (MESF) and different partitionates*i.e.* petroleum ether (PESF), carbon tetrachloride (CTCSF), and aqueous (AQSF) soluble partitionates to total phenolic content determination. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid (table 5.2) equivalents, results of the colorimetric analysis of the total phenolics are given in (table 5.3). Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of extractives.

The amount of total phenolic content differed in different extractives and ranged from 4 mg of GAE /gm of extractives to 25.43 mg of GAE /gm of extractives of *Ficusracemosa* Among all extractives of *Ficusracemosa* the highest phenolic content was found in CTCSF (77.63 mg of GAE /gm of extractives) followed by AQSF (49.44 mg of GAE /gm of extractives).Significant amount of phenolic compounds were also present in MESF (56.13 mg of GAE /gm of extractives), PESF (51.19 mg of GAE /gm of extractives) are also found.

Table-5.2: Standard curve preparation by using gallic acid.

x = GA Conc. (µg/ml)	y = Absorbance	Regression curve equation	R^2
100.000	1.620	y = 0.016x + 0.021	0.998
50.000	0.866		
25.000	0.450		
12.500	0.253		
6.250	0.120		
3.125	0.059		
1.563	0.034		
0.781	0.022		
0.391	0.02		
0.000	0.011		

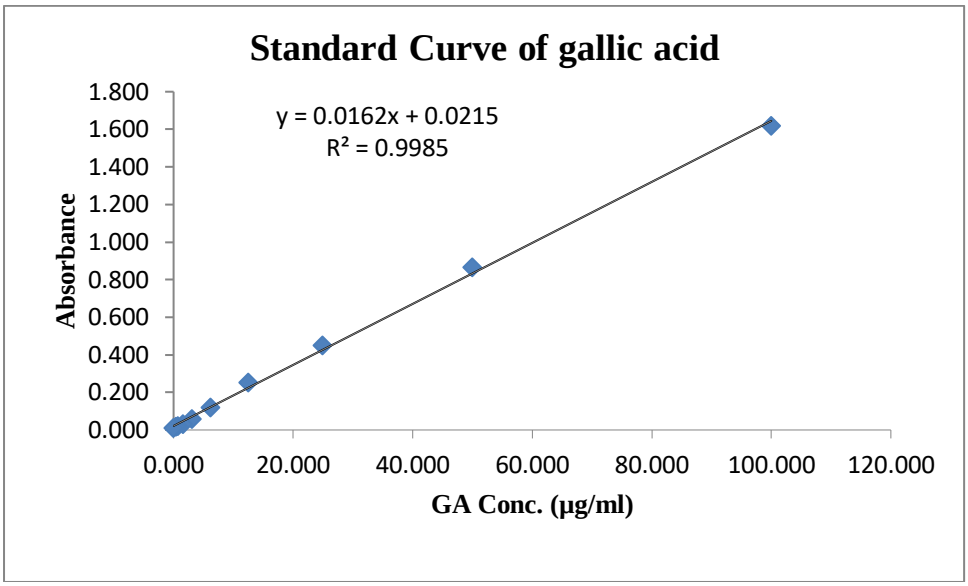


Figure: 5.3: Standard curve of gallic acid for total phenolic content determination (Author's own work).

Table-5.3: Test samples for Total phenolic content (TPC) determination

Plant part	Sample code	Test Sample	Total phenolic content (mg o GAE / gm of extractives
Fruits of*Ficusracemosa*	MESF	Methanol extract of the fruits of *Ficusracemosa*	56.13
	PESF	Petroleum ether soluble fraction	51.19
	CTCSF	Carbon tetrachloride soluble fraction	77.63
	AQSF	Aqueous soluble fraction	49.44

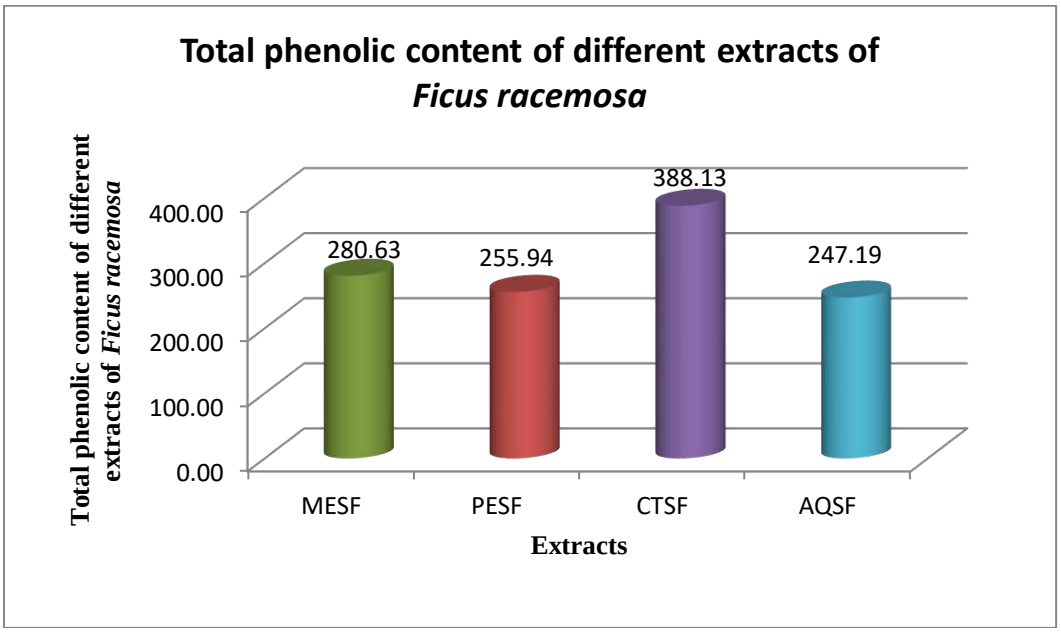


Figure: 5.4: Total phenolic content (mg of GAE / gm of extractives) of different extractives of leaf of *Ficusracemosa* (Author's own work).

Antimicrobial Activity:

6.1 Introduction

Infectious disease is one of main causes of death accounting for approximately one-half of all deaths in tropical countries worldwide. Perhaps, it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. Death from infectious disease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992; an increase of 58%. It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US[55]. This is alarming given that it was once believed that we would eliminate infectious disease by the end of the millennium. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most dramatic increases are occurring in the 25-44 years old age group[55].
These negative health trends call for a renewed interest in infectious disease in the medical, public health communities and renewed strategies on treatment and prevention. It is the last solution that would encompass the development of new antimicrobials [56]. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods[57].Disc diffusion method-

- ✓ Serial dilution method
- ✓ Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening [58]. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculums volume, culture medium composition, pH, and incubation temperature can influence the results [59]. Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity [59]. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method [60].

6.2 Principle of disc diffusion method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (Ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media [61]. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. 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 antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter [59], [61]. In the present study the crude extracts as well as fractions were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required [59].

6.3 Experimental

6.3.1 Apparatus and reagents

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

6.3. 2 Test organisms

The bacterial and fungal strains used for the experiment were collected as pure cultures from the State University of Bangladesh. Both gram positive and gram-negative organisms were taken for the test and they are listed in the table 6.1.

Table 6.1: List of organisms

Gram positive bacteria
Bacillus subtilis
Sarcina lutea
Staphylococcus aureus
Bacillus cereus
Gram negative bacteria
Escherichia coli
Shigella dysenteriae
Vibrio mimicus
Vibrio parahemolyticus
Salmonella typhi

6.3.3 Test materials

Table 6.2: List of Test materials

Plant part	**Sample code**	**Test Sample**
Flowers of *Ficusracemosa*	MESF	Methanolic extract soluble partitionate
	PESF	Petroleum ether soluble partitionate
	CTCSF	Carbon tetrachloride soluble partitionate
	AQSF	Aqueous soluble partitionate

6.3.4 Composition of culture medium

The following media was used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms.

Table: 6.3: List of culture medium

Composition of culture medium	
***a)* Nutrient ager medium**	
Ingredients	**Amount**
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s.	100 ml
Ph	7.2 + 0.1 at 250C

Nutrient agar medium is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

6.3.5 Preparation of the medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25^0C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure at 121^0C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

6.3.6 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. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light[62].

6.3.7 Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

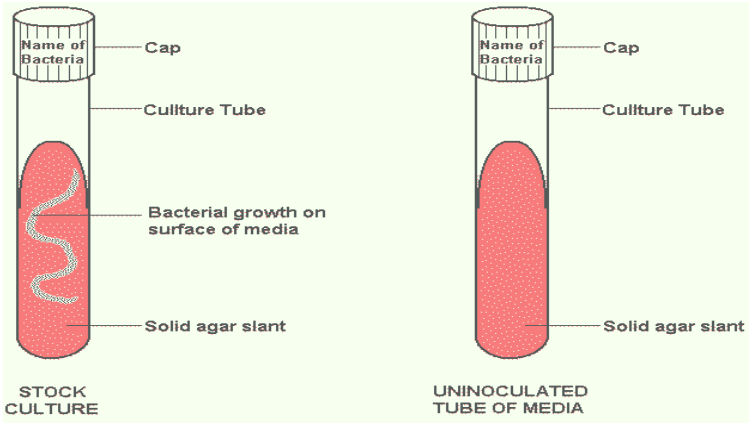


Figure:6.1: Preparation of subculture (source: microbenotes.com).

6.3.8 Preparation of the test plate

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

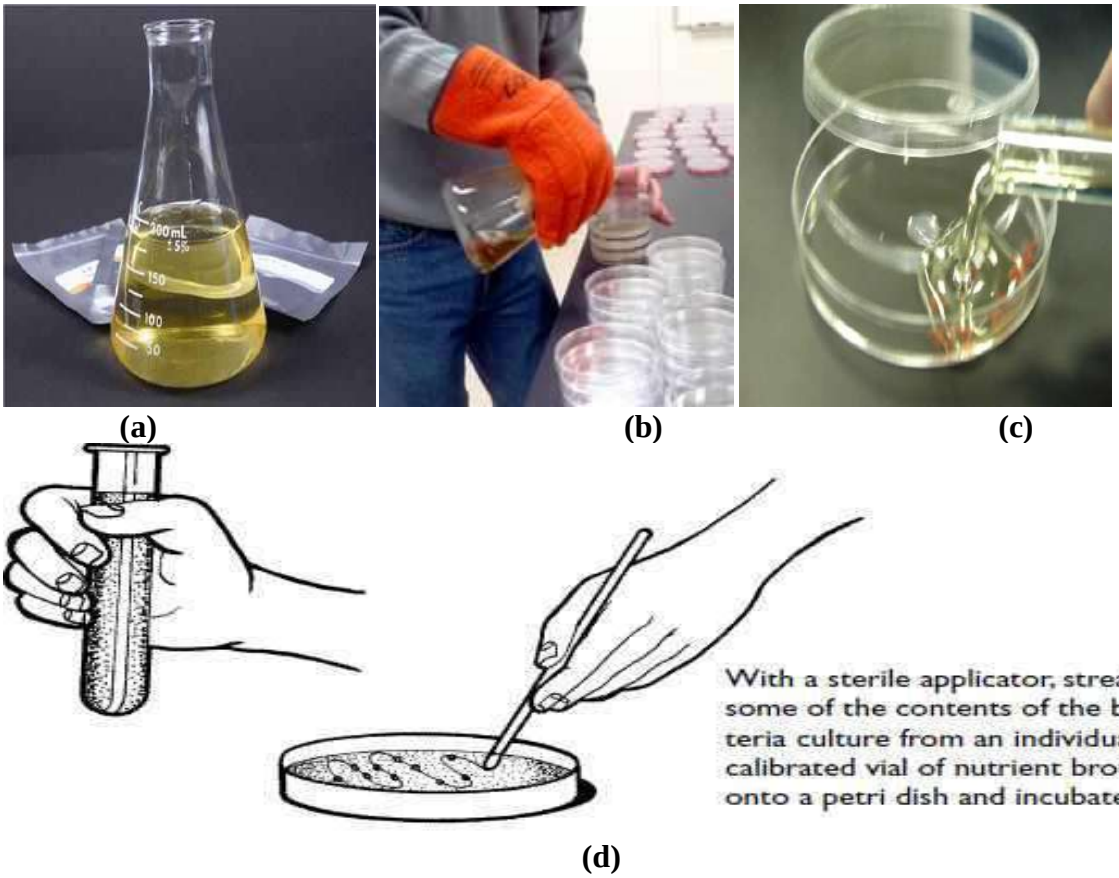


Figure: 6.2: (a) Preparation of the test plates (b) Freshly prepared culture medium, (c) Pouring culture medium to petridishes, (d) Transfer of bacterial and fungal suspension to the petridishes (Author's own work).

6.3.9 Preparation of discs

Measured amount of each test sample (specified in table 6.3) was dissolved in specific volume of solvent (Chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

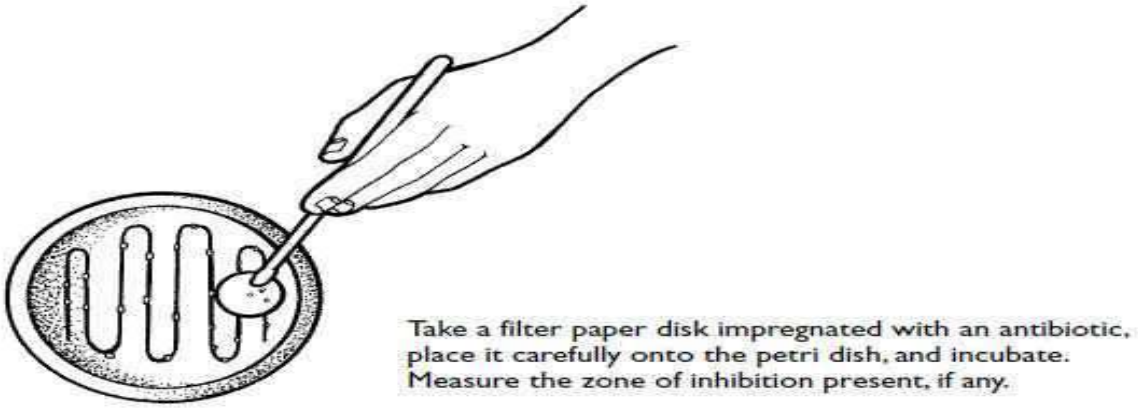


Figure 6.3: Preparation of filter paper discs (Author's own work).

Table 6.4: Preparation of sample Discs

Plant part	Test Sample	Dose µg /disc	Required amount for 20 disc (mg)
Flowers of *Ficusracemosa*	Methanol extract soluble fraction	400	8.0
	Pet-ether soluble fraction	400	8.0
	Carbon-tetrachloride soluble fraction	400	8.0
	Chloroform soluble fraction	400	8.0
	Aqueous soluble fraction	400	8.0

Standard Ciprofloxacin (50 µg/disc) 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. Blank 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.

6.3.10 Diffusion and 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 and fungi. The plates were then kept in a refrigerator at 4^0C 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 37^0C for 24 hours.

6.3.11 Determination of 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[63].

Figure 6.4: Clear zone of inhibition

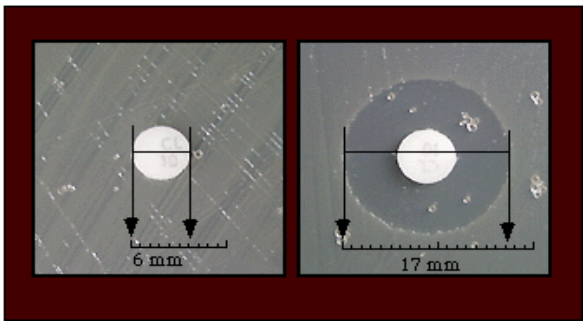


Figure 6.5: Determination of clear zone of inhibition (Author's own work)

6.4 Results and discussion of In vitro antimicrobial screening of *Ficusracemosa*

The Methanol extract of leaf of *Ficusracemosa* (MESF) and different partitionates i.e. Petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble partitionate of the methanol extract of leaf of *Ficusracemosa* were subjected to antimicrobial screening with a concentration of 400 μg/disc in every case. The results are given in the Table 6.5.

The Aqueous soluble fraction (AQSF) exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 11.0 mm. The maximum zone of inhibition produced by AQSF was found to be 11.0 mm against *Sarcina lutea*(+). This partitionate also showed moderate antibacterial activity against *Vibrio parahemolyticus(-), Escherichia coli(-),* having zone of inhibition of 9.0 mm. The AQSF also exhibited minimum inhibition against microbial growth *Staphylococcus aureus(-), Shigella dysenteriae*(-), having zone of inhibition 7.0 mm.

The Methanolic extract soluble fraction (MESF) also exhibited 2nd highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 9.0 mm. The maximum zone of inhibition produced by MESF was found to be 9.0 mm against *Salmonella typhi*(-), *Vibrio mimicus(-).*This partitionate also showed moderate antibacterial activity having zone of inhibition of 8.0 mm against *Bacillus cereus*(+), *Vibrio parahemolyticus*(-). The MESF also exhibited minimum inhibition against microbial growth *Sarcina lutea* (+), *Staphylococcus aureus*(+), *Shigella dysenteriae*(-) having zone of inhibition 7.50 mm.

The chloroform soluble fraction (CSF) exhibited the 3rd highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 9.0 mm. The maximum zone of inhibition produced by CSF was found to be 9.0 mm against *staphylococcus aureus*(+). This partitionate also showed moderate antibacterial activity having zone of inhibition of 8.0 mm against *Sarcina lutea, Bacillus cereus*(+),*Shigella dysenteriae*(-), *Vibrio mimicus*(-).The CSF also exhibited minimum inhibition against microbial growth *Vibrio parahemolyticus*(-) having zone of inhibition 7.50 mm.

The Petroleum ether soluble fraction (PESF) exhibited 4th highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 8.0 mm. The maximum zone of inhibition produced by PESF was found to be 8.0 mm against *Sarcina lutea*(+), *Escherichia* coli(-), Vibrio*mimicus*(-), *Salmonella typhi*(-). The PESF also exhibited minimum inhibition against microbial growth *Bacillus subtilis*(+), *Staphylococcus aureus*(+),*Vibrio parahemolyticus*(-) having zone of inhibition 7.00 mm.

The Carbon tetra-chloride soluble fraction (CTCSF) exhibited the 5th highest inhibition against microbial growth having zone of inhibition ranged from 8.0 mm to 7.0 mm. The maximum zone of inhibition produced by CTCSF was found to be 8.0 mm against *Sarcina*

lutea(+),*Vibrio mimicus*(-). This partitionate also showed moderate antibacterial activity against *Salmonella typhi Staphylococcus aureus*(+), *Bacillus cereus*(+), *Shigella dysenteriae*(-), having zone of inhibition of 7.5.0 mm and exhibited minimum inhibition against microbial growth *Staphylococcus aureus*(+),*Bacillus cereus*(+),*Shigella dysenteriae*having zone of inhibition 7.0 mm.

The results of *in-vitro* microbial screening of flowers of *Ficusracemosa* indicated that AQSF, MESF, CSF, PESF and CTCSF, possess better antimicrobial activity and these can be further studied to explore potent antimicrobial agents. Besides all these test subjects have significant activity against gram positive and gram negative bacteria.

Table 6.5: Antimicrobial activity of test samples of *Ficusracemosa*

Test M.O	**Diameter of zone of inhibition (mm)**					
	MESF	**PESF**	**CTCSF**	**CSF**	**AQSF**	**Ciprofloxacin (Standard)**
Gram positive bacteria						
Bacillus subtilis	**-**	**7.00**	**-**	**7.00**	**-**	**37.00**
Sarcina lutea	**7.00**	**8.00**	**8.00**	**8.00**	**11.00**	**36.00**
Staphylococcus aureus	**7.00**	**7**	**7.00**	**9.00**	**7.00**	**42.00**
Bacillus cereus	**8.00**	**-**	**7.00**	**8.00**	**-**	**37.00**
Gram negative bacteria						
Escherichia coli	**-**	**8.00**	**-**	**7.00**	**9.00**	**38.00**
Shigella dysenteriae	**7**	**-**	**7.00**	**8.00**	**7.00**	**39.00**
Vibrio mimicus	**9.00**	**8.00**	**8.00**	**8.00**	**-**	**40.00**
Vibrio parahemolyticus	**8.00**	**7.00**	**-**	**7.50**	**9.00**	**38.00**
Salmonella typhi	**9.00**	**8.00**	**7.50**	**-**	**-**	**38.00**

Membrane Stabilizing Activity:

7.1 Introduction

In many of the pathological disorders, inflammation is the one of the important processes. Inflammatory cells produce a complex mixture of growth and differentiation of cytokines as well as physiologically active arachidonate metabolites. In addition they possess the ability to generate reactive oxygen species (ROS) that can damage cellular biomolecules which in turn augment the state of inflammation[64].Compounds that possess radical scavenging ability may therefore expect to have the therapeutic potentials for inflammatory disease[65].The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane[40].Therefore, as membrane stabilizes that interfere in the release and or action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc[66].Thus, the aim of the present study was to investigate the anti-inflammatory activity of methanolic extract and its different fractions of fruits of *Ficusracemosa.*

7.2 Materials and method

7.2.1 Preparation of the extract

Table 7.1:Preparation of different extracts of roots of *Ficusracemosa*

Sample code	Concentration
Hypotonic solution	2mg/ml
MESF	2mg/ml
PESF	2mg/ml
CTCSF	2mg/ml
CSF	2mg/ml
AQSF	2mg/ml
ASA (Acetyl Salicylic Acid)	0.1mg/ml

Solvent used: Methanol analytical grade

7.2.2 Drug

Standard Acetyl Salicylic Acid (ASA) or Aspirin was used as standard drug for comparison with different methanolic extracts of roots of *Ficusracemosa.*

7.2.3 Red Blood Cells (RBC) collection

Human RBCs were collected for the study. RBCs collected from the human was male, 70 kg, fare complexion and free from diseases. The collected RBCs were kept in a test tube with an anticoagulant EDTA under standard conditions of temperature 23±2°C and relative humidity 55±10%.

7.2.4 Preparation of Phosphate buffer solution

A buffer is an aqueous solution that has a highly stable pH. The buffer was prepared at pH 7 using monosodium phosphate and its conjugate base, disodium phosphate.

7.2.4.1 Phosphate buffer materials

- Monosodium phosphate
- Disodium phosphate
- Water
- pH meter
- Glassware
- Stirring bar

7.2.4.2 Calculation of Phosphate buffer

A pH of about 7.4 with buffer strength of 10 mM was obtained using 0.0352% monosodium phosphate dehydrate and 0.1099% disodium phosphate anhydrate. The buffer was made by

adding 0.352 gm monosodium phosphate dehydrate and 1.099 gm disodium phosphate anhydrate to 1000 mL water.

- pH: 7.4
- Buffer strength: 10.00 mM
- Monosodium phosphate, dehydrate: 0.0352%
- Disodium phosphate, anhydrate: 0.1099%

7.2.5 Preparation of isotonic solution

A solution that has a concentration of electrolytes, nonelectrolytes or a combination of the two that will exert equivalent osmotic pressure as that solution with which it is being compared.

Either 0.16M sodium chloride (NaCl) solution (approximately 0.95% salt in water) or 0.3M non-electrolyte solution is approximately isotonic with human red blood cells.

For the preparation of 500 ml isotonic solution of 154 mM strength, 4.5045 gm NaCl was added and mixed.

7.2.5.1: Material for isotonic solution

- Sodium chloride (NaCl)
- Water
- Glassware
- Stirring bar

7.2.5.2 Calculation for isotonic solution

1000 ml solution of strength 1 M contain = 58.5 gm NaCl

500 ml solution of strength 1 M contain = 58.5/2 gm NaCl

500 ml solution of strength 1000 mM contain = 58.5/2 gm NaCl

500 ml solution of strength 154 mM contain = 58.5 × 154/2 × 1000 gm NaCl

= 4.5045 gm NaCl

7.2.6 Preparation of hypotonic solution

A solution of lower osmotic pressure than that of a reference solution or of an isotonic solution is called hypotonic solution.

For the preparation of 500 ml hypotonic solution, having strength of 50 mM, 1.4625 gm NaCl was added and mixed.

7.2.6.1 Materials for hypotonic solution

- Sodium chloride (NaCl)
- Water
- Glassware
- Stirring bar

7.2.6.2 Calculation for hypotonic solution

1000 ml solution of strength 1 M contain = 58.5 gm NaCl

500 ml solution of strength 1 M contain = 58.5/2 gm NaCl

500 ml solution of strength 1000 mM contain = 58.5/2 gm NaCl

500 ml solution of strength 50 mM contain = 58.5 × 50/2 × 1000 gm NaCl

= 1.4625 gm NaCl

7.2.7 Effect on haemolysis

7.2.7.1 Erythrocyte suspension

Whole blood was collected from male human under standard condition. EDTA was used to prevent clotting. The blood was washed three times with isotonic solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifuge action for 10 min at 3000 rpm. Thus the suspension finally collected was the stock erythrocyte (RBC) suspension.

7.2.7.2 Hypotonic solution Induced hemolysis

The experiment was carried out with hypotonic solution. The test sample consisted of stock erythrocyte (RBC) suspension(0.5ml) with 5ml of hypotonic solution(50mm NaCl) in 10 mm sodium phosphate buffer saline (pH 7.4) containing either the different methanolic extract (2mg/ml) or acetyl salicylic acid (.1mg/ml). The acetyl salicylic acid was used as reference standard. The mixture was incubated for 10 min of room temperature centrifuge for 10 min of 3000 RPM and the absorbance of the separated was measured of 540nm using shimadzu UV spectrophotometer.

The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation.

% inhibition of haemolysis $=100\times \{(OD_1-OD_2)/OD_1\}$

Where,

OD_1= optical density of hypotonic buffered saline solution alone (control) and

OD_2= optical density of the test sample in hypotonic solution.

7.3 Result and discussion of the test sample of *Ficusracemosa*

The different methonolic extracts of the fruits of concentration 2.0 mg/ml significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution as compared to the standard acetyl salicylic acid.

The roots of *Ficusracemosa* were effective in the membrane stability activity as the extractive prevented the lysis of erythrocyte induced by hypnotic solution. The methanolic extract (MESF) inhibited 39.84%, petroleum ether soluble fractionate(PESF) inhibited 19.70%, carbon tetrachloride soluble fractionate (CTCSF) inhibited 46.63% and aqueous soluble fractionate (AQSF) inhibited 57.85 % for membrane stabilizing activity acetyl salicylic acid was used as standard drug that exhibited 75.76 % inhibition of haemolysis of normal condition.

Table 7.2:Effect of different extractions of Fruits of *Ficusracemosa* of hypnotic solution induced haemolysis of erythrocyte membrane.

Sample Code	Concentration	Absorbancce	% Inhibition
Hypotonic	50 mm	1.675	0
MSF	2 mg/ml	1.125	32.84
PESF	2 mg/ml	1.345	19.70
CTSF	2 mg/ml	0.894	46.63
AQSF	2 mg/ml	0.706	57.85
ASF	2 mg/ml	0.406	75.76

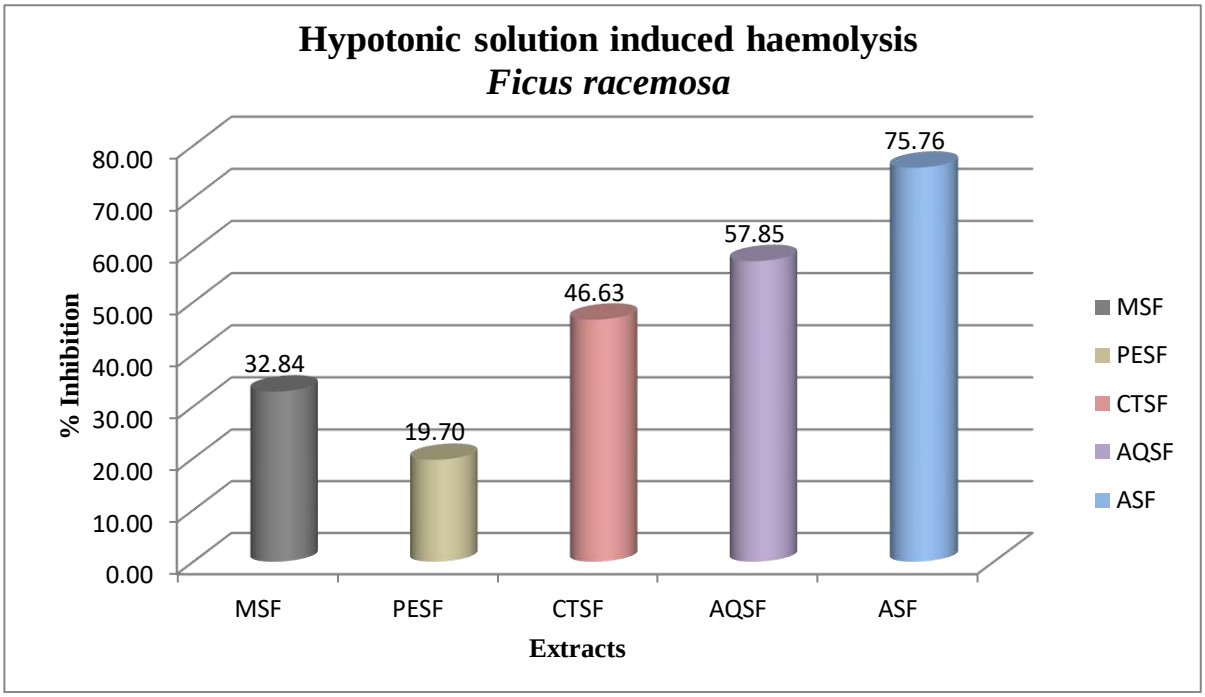


Figure 7.1: Hypotonic solution induced haemolysis (Author's own work)

7.3.1 Heat induced haemolysis

Aliquots of the isotonic buffer containing 2 mg/ml of different extractive of *Ficusracemosa* were put into two duplicates of centrifuging tube[66].The vehicle in the same amounts was added to another tube as control. Erythrocyte suspension (30 µl) was added to each tube and mixed gently by inversion. One pair of tubes was incubated at 54^0C for 20 min in a water bath. The other pair was maintained of 0-5ºC in an ice bath. The reaction mixture was centrifuged for 10 min at 1300 RPM and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation.

$$\% \text{ inhibition of haemolysis} = 100\times \{1-\times\frac{OD2-OD1}{OD3-OD1}\}$$

$$=100\times \{\frac{OD3-OD1-OD2+0D1}{OD3-OD1}\}$$

$$=100\times\frac{OD3-OD2}{OD3-)D1}$$

Where,
OD_1= test sample unheated
OD_2= test sample heated and
OD_3= control sample

The different methonolic extracts of the roots of concentration 2.0 mg/ml significantly protected the lysis of human erythrocyte membrane induced by heat as compared to the standard acetyl salicylic acid.

The fruits of *Ficusracemosa* were effective in the membrane stability activity as the extractive prevented the lysis of erythrocyte induced by hypnotic solution. The methanolic extract (MESF) inhibited 67.89 %, petroleum ether soluble fractionate (PESF) inhibited 63.10 %, carbon tetrachloride soluble fractionate (CTCSF) inhibited 69.08 % and aqueous soluble fractionate (AQSF) inhibited 70.78% for membrane stabilizing activity acetyl salicylic acid was used as standard drug that exhibited 85.41 % inhibition of haemolysis of normal condition.

Table 7.3: Effect of different extraction of fruits of *Ficusracemosa* of heat induced haemolysis of erythrocyte membrane.

Sample Code	Concentration	Unheated(OD1)	Heated(OD2)	OD3-OD2	OD3-OD1	% Inhibition
Hypotonic	50 mm	3.971(OD3)				
MSF	2 mg/ml	0.433	1.569	2.402	3.538	67.89
PESF	2 mg/ml	0.532	1.801	2.17	3.439	63.10
CTSF	2 mg/ml	0.278	1.42	2.551	3.693	69.08
AQSF	2 mg/ml	0.648	1.619	2.352	3.323	70.78
ASA	0.10 mg/ml	1.16	1.57	2.401	2.811	85.41

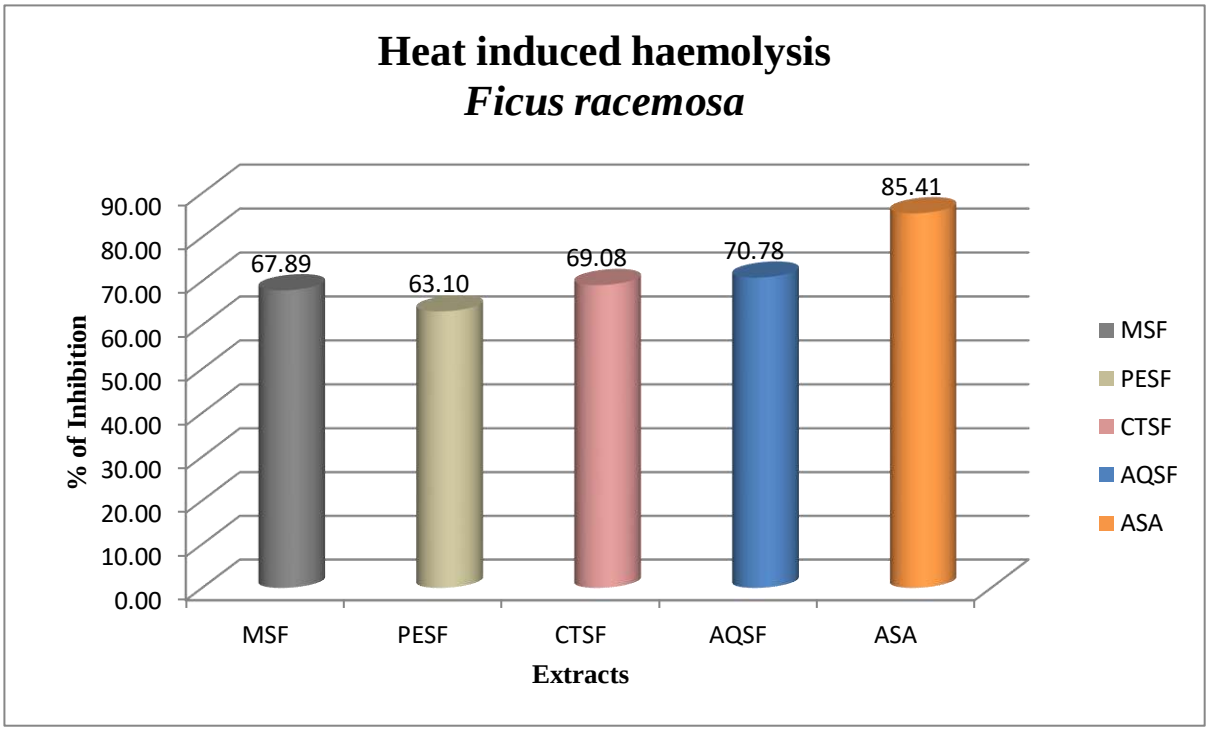


Figure 7.2: Heat induced haemolysis (Author's own work)

The effect of synthetic and herbal anti-inflammatory agents on the stabilization of erythrocyte membrane exposed to hypotonic solution has been studied extensively. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane.
The results showed that the extracts were potent on human erythrocyte adequately protecting it against hypotonic induced lysis. The activity was comparable to that of standard anti-inflammatory drug (Acetyl Salicylic Acid). It has been reported that flavonoids exert profound stabilizing effects on lysosomes both in vitro and in vivo experimental animals while tannin have the ability to bind cations and other bio-molecules, and are able to stabilize erythrocyte membrane.
The present investigation suggests that the membrane stabilizing activity of root of *Ficusracemosa* plays a significant role in its anti-inflammatory activity may be due to its high flavonoids and tannin content.

Evaluation of thrombolytic Activity:

8.1 Introduction

Since ancient periods, herbal preparations have been used for the treatment of several diseases. The leaves and/or twigs, stem, bark and underground parts of plants are most often used for traditional medicines. Herbal products are often perceived as safe because they are "natural"[67].Cerebral venous sinus thrombosis (CVST) is a common disorder which accompanied by significant morbidity and mortality[67].Heparin, an anti-coagulating agent, is the first line of treatment for CVST, because of its efficacy, safety and feasibility. [69] Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of patients with CVST[70].Thus, the aim of the present study was to investigate the thrombolytic activity of methanolic extracts and its different fractions of roots of *Ficusracemosa.*

8.2 Materials & Methods

8.2.1 Preparation of sample

The thrombolytic activity of all extractives was evaluated by a method using streptokinase (SK) as standard substance. 10 mg of methanolic extracts and its different fractions of whole plant of *Ficusracemosa or* were taken in different vials to which 1ml distilled water was added.

8.2.2 Streptokinase (SK)

Commercially available lyophilized Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolytic.

8.2.3 Blood Sample

Whole blood (n=10) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed sterile vials and was allowed to form clots.

8.2.4 Thrombolytic activity

Aliquots (5 ml) of venous blood were drawn from healthy volunteers who were distributed in ten different pre weighed sterile vials (1 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each vial having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone).

To each vial containing pre-weighed clot, 100 µl aqueous solutions of

Ficusracemosa different partitionates along with the crude extracts was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control vials. All the vials were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released of fluid was removed and vials were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot weight) × 100

8.3 Results and Discussion of thrombolytic activity of *Ficusracemosa*

As a part of discovery of cardio protective drugs from natural sources the extractive of the leaf either assess or not for thrombolytic activity and results are presented in the table (8.1). Addition of 100M1SK, a positive control (30000.00) to the clot and subsequent incubation for 90minutes at 37°c showed 65.69% lysis of clot on the other hand distilled water was

treated as negative control which exhibited (10.23%) a negligible percentage between positive and negative control was found very significant.

In this study, the thrombolytic activity, among all extractives of rots of *Ficusracemosa* was found to be negligible. The amounts of thrombolytic activity were present in Methanol soluble fraction (MESF 32.91%), Pet-ether soluble fraction (PESF 34.27%), carbon tetra chloride soluble fraction (CTCSF 30.70%) and dichloromethane soluble fraction (CSF 38.83%), and Aqueous soluble fraction (AQSF 31.18%).

Table 8.1: Thrombolytic Activity (in terms of % of clot lysis) of the extractives of *Ficusracemosa.*

Fractions	Weight of empty vial W_1 gm	Weight of vial with clot W_2 gm	Weight of clot $W_3 = W_2 - W_1$ gm	Weight of vial after clot lysis W_4 gm	Weight of lysis clot $W_5 = W_2 - W_4$ gm	%of clot lysis= 100 X W_5/W_3
MESF	**5.05**	**7.42**	2.37	**6.64**	0.78	32.91
PESF	**5.10**	**7.23**	2.13	**6.50**	0.73	34.27
CTSF	**5.07**	**7.22**	2.15	**6.56**	0.66	30.70
CSF	**4.50**	**5.53**	1.03	**5.13**	0.40	38.83
AQSF	**5.15**	**6.85**	1.70	**6.32**	0.53	31.18
Blank	**5.26**	**7.12**	1.86	**6.93**	0.19	10.23
SK	**5.34**	**7.25**	1.90	**6.00**	1.25	65.69

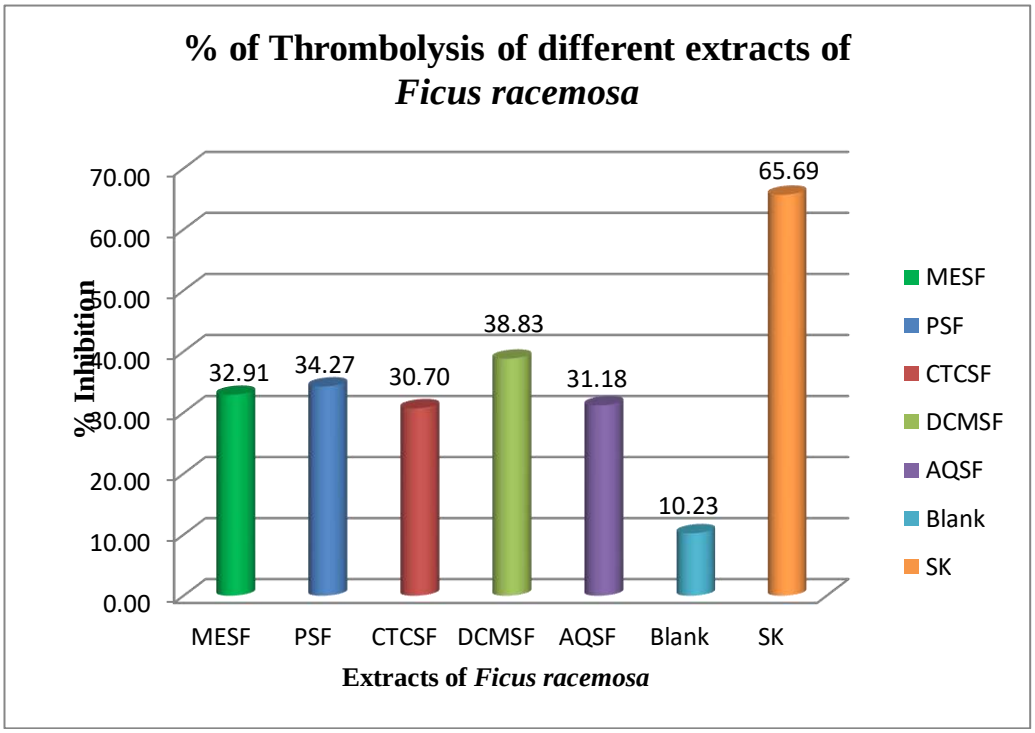


Figure 8.1: Thrombolytic Activity (in terms of % of clot lysis) of extractives of *Ficusracemosa* (Author's own work).

From this experiment, it can be concluded that the extractives of *Ficusracemosa* showed less clot lysis activity than the standard substance streptokinase (SK).

Evaluation of peripheral Analgesic Activity:

9.1 Introduction

Inflammation is the response of a tissue and its microcirculation to pathogenic injury. It is characterized by the generation of inflammatory mediators and movement of fluid and leucocytes from the blood into extra vascular tissues which gives rise to the four cardinal signs of inflammation, namely rubor (redness), calor (heat), tumor (swelling) and dolor (pain) as described by AnlusCelsus, the Roman encyclopedist, in the second century AD. Inflammation may be classified into acute, sub-acute and chronic or immunological. There are various mediators for these types of inflammation in different stages, which are histamine, 5-hydroxytryptamine, bradykinin, prostaglandins, leukotrienes, etc

9.2 Principle

Peripheral analgesic activity can be evaluated by acetic acid induced writhing method[71].In this method acetic acid is administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Diclofenac was used as a standard drug[72].According to this principle the crude methanolic extract of roots of *Fecusracemosa* was subjected to analgesic testing at two different doses: Dose 200 mg/kg and 400 mg/kg of body weight.

9.3 Experimental Animal

Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDRB) were used for the experiment. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 h light-dark cycle and fed ICDDR; B formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Figure 9.1: Swiss albino mice

Figure 9.2: Oral administration (Author's own work)

9.4 Experimental Design

Twelve experimental animals were randomly selected and divided into four groups denoted as group-I, group-II and group-III (A-B) consisting of 3 mice in each group. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. As it was difficult to observe the biologic response of three mice at a time receiving same treatment, it was necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way (Figure 11.3) and marked as M-1= Mice 1, M-2= Mice 2, M-3= Mice 3.

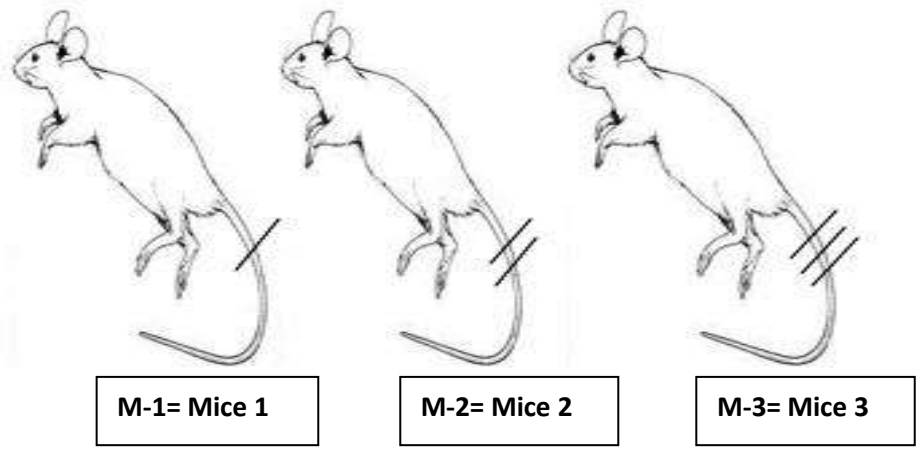


Figure 9.3: Numbering of mice (Author's own work)

9.4.1 Preparation of Test Materials

In order to administer the extract at doses of 400 mg/kg body wt and 200 mg/kg body wt of mice, the exactly weighed extracts were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Diclofenac at the, 50 mg of diclofenac was taken and a suspension of dose of 50 mg/kg-body weight was made.

9.4.2 Procedure

At zero hour test samples, control (1% Tween-80 solution in saline) and Diclofenac sodium were administered orally by means of a long needle with a ball-shaped end.

⇩

0.1 ml 1% glacial acetic acid (1 ml glacial acetic acid diluted to 100 ml distilled water) was injected after 30 minutes of administration of Diclofenac-Na and test samples to each of the animals of all the groups

⇩

Responses were measured during 10 minute after the first 5 minutes of acetic acid injection

Figure 9.4: Schematic representation of procedure for screening of analgesic property

9.4.3 Counting of licking and biting responses

Each mouse of all groups were observed individually for counting the number of licking and biting responses they made in 5 minutes commencing just after the subcutaneous administration of formalin solution.

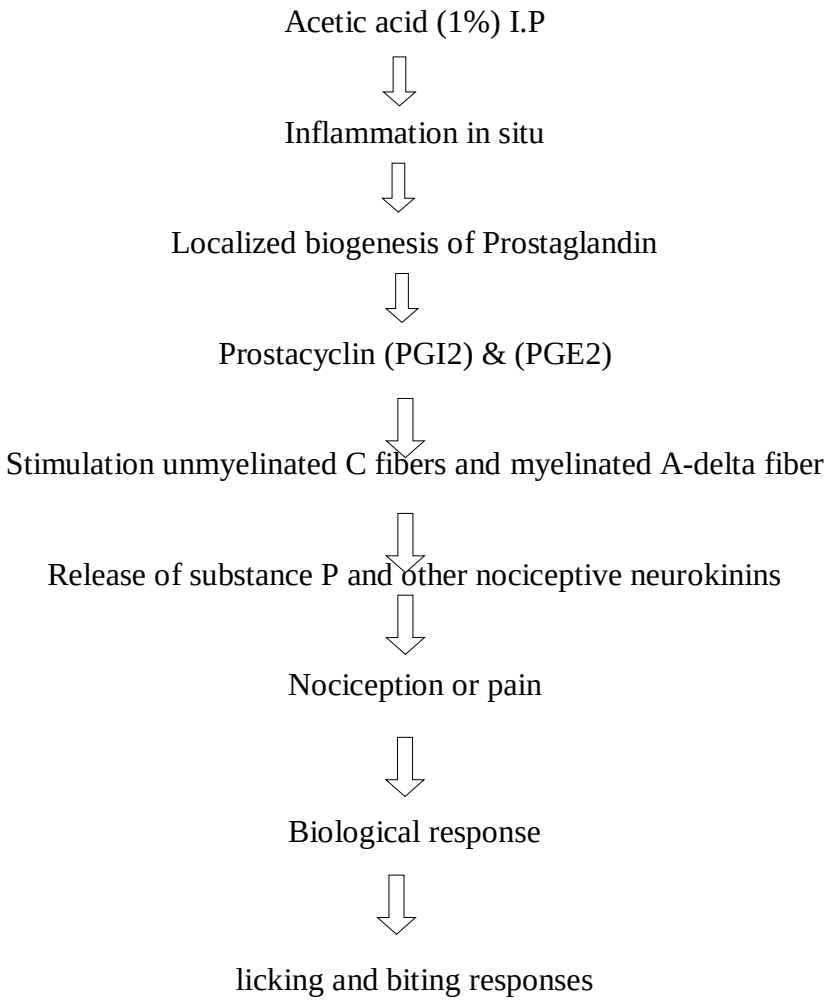


Figure 9.5: Mechanism of action of pain induction (Author's own work).

9.5.1 Data collection

Table 9.1: Test materials used in the evaluation of analgesic activity of flowers of *Fecusracemosa.*

Code no.	Test Samples	Group	Identification	Dose (mg/kg)*
CTL	1% Tween-80 in normal saline	I	Control Group	0.1 ml/10 g of body wt
STD	Diclofenac sodium	II	Standard Group	50
MESF 1	Methanolic extract	III A	Test Sample	200
MESF 2	Methanolic extract	III B	Test Sample	400

*All doses were given orally. 0.1 ml of 1% acetic acid solution was injected into the peritonial to each mouse.

Table 9.2: Screening of analgesic activity by counting the time of licking and biting after subcutaneously administration of 5% acetic acid

Animal Group	Writhing Count			Average	Standard Deviation	Standard Error	Writhing (%)	% Inhibition
	M-1	M-2	M-3					
Control (H_2O)	17	12	16	15	1.24	0.88		-
Standard (Diclofenac)	3	2	4	3	0.471	0.33		80
MESF (200 mg)	5	4	6	5	0.471	0.33		66.66
MESF (400 mg)	3	6	4	4.33	0.72	0.50		71.13

In the table M-1 = mice 1, M-2 = mice 2 and M-3 = mice 3.

According to the data that the above table, the Methanolic extracts of leaf of *Fecusracemosa* at dose of 400 mg/kg exhibited significant peripheral analgesic activity and at doses of 200mg/kg also showed peripheral analgesic activity to a lesser extent compared to Diclofenac Na.

Table 9.3: Statistical evaluation for the peripheral analgesic activity test

Code no.	Writhing (%)	Inhibition (%)	t value	P value	Level of significance
Standard (Diclofenac)	-	80	11.0322	.004058	Very statistically significant

MESF (200 mg)	-	66.66	18.3870	.001472	Very statistically significant
(MESF 400 mg)	-	71.13	10.4164	.004546	Very statistically significant

Statistical evaluation of the data obtained after the administration of the samples confirmed that the Methanolic extract of roots of *Fecusracemosa* at doses of 200 mg/kg and 400 mg/kg were showed a percent Inhibition of 4.55% and 9.09% respectively. Both data was extremely statistically significant.

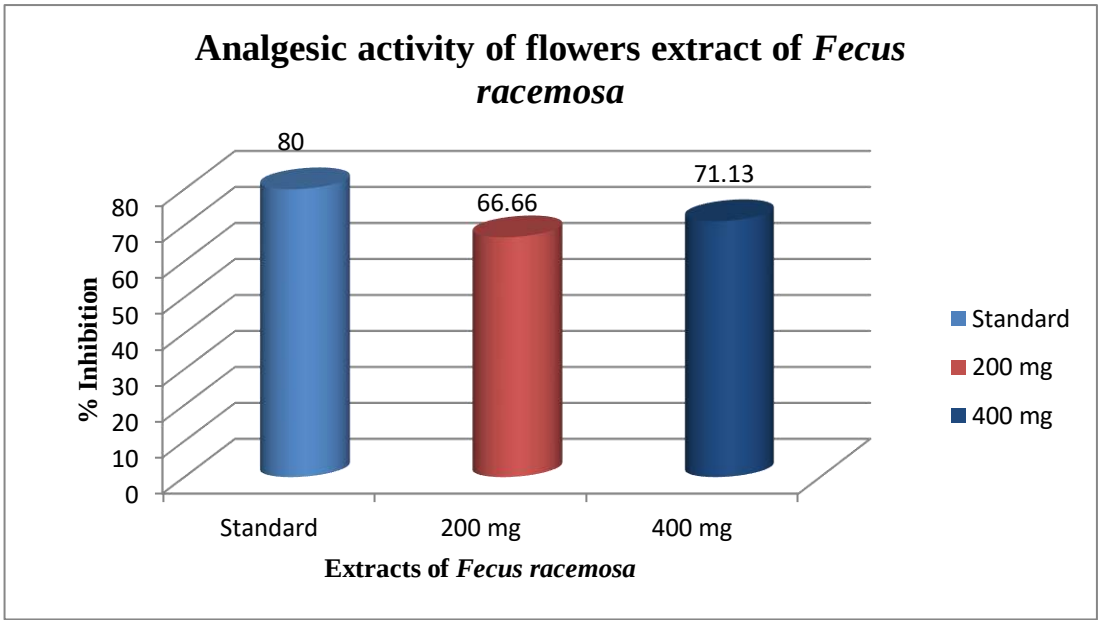


Figure 9.6: Comparison of %inhibition of licking responses by Methanolic extract of *Fecusracemosa* (Author's own work)

9.5 Result and Discussion

The different test samples were subjected to screening for analgesic activity by acetic acid test. The test was performed by taking samples at doses 200 mg/kg and 400 mg/kg body weight. The result was statistically evaluated and the t-test and p values were determined. All the test materials exhibited highly significant peripheral analgesic activity at both the two doses while the crude extract at 400 mg/kg dose exhibited maximum inhibition of licking and biting71.13%.

Evaluation of central Analgesic Activity:

10.1 Principle

Evaluation of central analgesic activity was carried by tail immersion method using Morphine as a positive control. The changes in sensitivity of test animal due to analgesic activity of drugs are measured in this method. A constant heat stress is applied to rat tail, which acts as pain stimulus. When the stimulus exceeds the threshold, rat show a quick withdrawal of its tail. Time taken by the rat to withdraw the tail is termed as tail immersion time. Analgesic compound elongates this responding time. By this test discrimination was done between centrally acting morphine –like analgesics and non-opiate analgesics. The test rats were orally fed with test materials whereas the positive control received morphine subcutaneously. From 1-2 cm of the tail of mice was immersed in warm water kept constant at 55^0 C .The reaction time is the time required by the mice to deflect their tails. The time required to withdraw the tail was recorded.

10.2 Experimental animal

Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 h light-dark cycle and fed ICDDR,B formulated rodent food and water (ad-libitum). As these animals were very sensitive to environmental changes, they were kept before the test for at least 3-4 days in the environment where the experiment will take place. Food was withdrawn 12 hours before and during the experiment. The ethics for use of experimental animals were followed carefully.

Figure 10.1: Swiss albino mice

10.3 Experimental Design

Fifty experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III(A-B), group- IV(A-B), consisting of 3 mice in each group. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. As it was difficult to observe the biologic response of three mice at a time receiving same treatment, it was necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way (Figure 12.2) and marked as M1=Mice 1, M2=Mice 2, and M3=Mice 3.

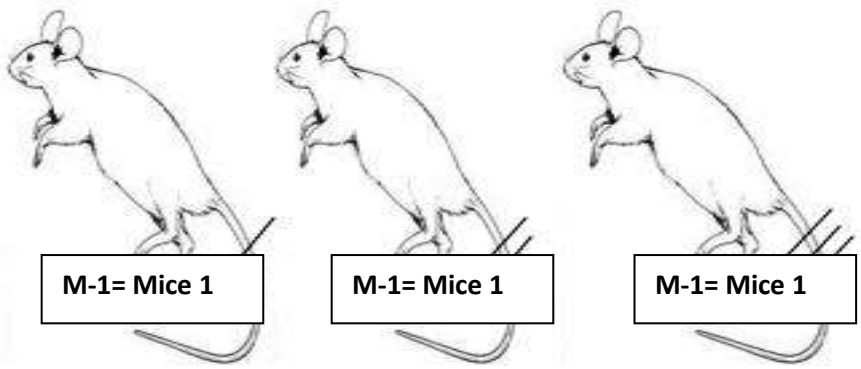


Figure 10.2: Numbering of mice (Author's own work)

10.3.1 Materials and equipments

- Mice holder
- Feeding needle
- Syringe
- Morphine
- Mice cage

10.4 Experimental Procedure

10.4.1 Preparation of Test Materials

In order to administer the extract at doses of 400 mg/kg body wt and 200 mg/kg body wt of mice, the exactly weighed extracts were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture.

Diclofenac-Na (Clofenac Plus of Square Pharmaceuticals Ltd) was administered orally in the form of solution. The solution was prepared by diluting the supplied diclofenac-Na (4 mg in 10 ml) with saline water. Then diclofenac-Na which ensured a dose of 2mg/kg for 32 mg mice.

10.4.2 Methodology

Test samples and control were given orally by means of a feeding needle to the mice at zero hour. At zero hour, 1-2 cm of the tail of mice was immersed in warm water kept constant at 55^0 C. The reaction time is the time required by the mice to deflect their tails. The first reading is discarded and the reaction time is recorded as a mean of the next three reading. A latency period of 20 seconds was defined as complete analgesia and the measurement was stopped to avoid injury to mice. The latent period of tail-flick response was determined before and 0, 30, 60 and 90 minutes after the administration of drugs. A 30 minutes interval was given to ensure proper absorption of the administered substances. Then diclofenac-Na solution was administered subcutaneously to the mice. After 30 minutes, 60 minutes and 90 minutes, the tail immersion time was measured.

The time for tail immersing of each mouse was recorded and the average immersing time of each group was calculated. The % time elongation of tail immersing was calculated in respect

to the control. The higher the elongation percentage of the group the greater is the group's central analgesic activity. The central analgesic activity of the test samples were compared in respect to diclofenac-Na.

$$\%\text{Time elongation} = \frac{\text{Average time of tail flicking of test samples - Average time of tail immersing of the control group}}{\text{Average time of tail flicking of control group}} X100\%$$

Table 10.1: Test materials used in the evaluation of central analgesic activity of crude extract and its different fractions of *Ficusracemosa.*

Code no.	Test Samples	Group	Identification	Dose (mg/kg)*	Route of administration
Control	Distilled Water	I	Control Group	50 ml	Oral
Standard	Diclofenac Na	II	Standard Group	2 mg	Oral
MESF 200mg	Methanolic extract	III A	Test Sample	200 mg	Oral
MESF 400mg	Methanolic extract	III B	Test Sample	400 mg	Oral

Table 10.2: Analysis of the data obtained after 30 minutes

Animal Group	Immersion time count (seconds)			Average time of immersion	Standard deviation	Standard deviation error	% Elongation
Control (Water)	M-1	M-2	M-3	2.65	0.14	0.099	-
	2.62	2.56	2.78				
Standard (Diclofenac)	4.43	4.69	5.32	4.81	0.215	0.152	81.50
MESF (200 mg)	2.85	3.79	2.94	3.19	0.285	0.20	20.30
MESF (400 mg)	3.96	4.53	3.63	4.04	0.214	0.151	52.45

According to the data in the above table the methanol soluble fraction at dose of 400mg/kg exhibited highly significant central analgesic activity. The crude extracts at doses of 400mg/kg also exhibited significant central analgesic activity after 30 minutes compared to diclofenac Na. The MESF at doses of 200mg/kg also showed central analgesic activity to a lesser extent.

Table10.3: Statistical Evaluation of the data obtained after 30 minutes

Group	t-Test value	Degree of freedom	P value	Level of significance
Standard (Diclofenac)	38.7496	2	0.000333	Extremely statistically significant

MESF (200 mg)	19.3868	2	0.001325	Very Statistically significant
MESF (400 mg)	32.6985	2	0.000467	Extremely statistically significant

Statistical evaluation of the data obtained 30 minutes after the administration of the samples confirmed that the methanolic extract at doses of 400 mg/kg exhibited significant central analgesic activity. The crude at doses of 200 mg/kg showed central analgesic activity to a lesser extent.

Table 10.4: Analysis of the data obtained after 60 minutes

Animal Group	Immersion time count (seconds)			Average time of immersion	Standard deviation	Standard deviation error	% Elongation
Control (Water)	M-1	M-2	M-3	2.60	0.184	0.130	-
	2.97	2.19	2.64				
Standard (Diclofenac)	3.79	4.88	4.56	4.39	0.264	0.186	68.84
MESF 200 mg	3.87	3.14	3.10	3.37	0.204	0.144	29.61
MESF 400 mg	3. 50	4. 70	3.95	4.05	0.285	0.201	55.77

In the table M-1 = mice 1, M-2 = mice 2, M-3 = mice 3

The data in Table: 11.4 showed that after 60 minutes the central analgesic action of Crude Methanol extract at a dose of 400 mg/kg became stronger. They showed a percent elongation of 55.77% and 29.61% at a dose of 400 mg and 200 mg/kg bode weight. But the central analgesic activity of aqueous soluble fraction at 400 mg/kg dose still remains highly significant after 60 minutes.

Table 10.5: Statistical Evaluation of the data obtained after 60 minutes

Group	t-Test value	Degree of freedom	P value	Level of significance
Standard (Diclofenac)	28.8019	2	0.000602	Very statistically significant
MESF (200 mg)	28.6128	2	0.00061	Very statistically significant
MESF (400 mg)	24.6134	2	0.000823	Very statistically significant

Table 10.6: Analysis of the data obtained after 90 minutes

Animal Group	Immersion time count (seconds)			Average time of immersion	Standard deviation	Standard deviation error	% Elongation
Control (Water)	M-1	M-2	M-3	2.35	0.225	0.159	-
	1.93	2.87	2.25				

Standard (Diclofenac)	4.78	3.82	4.81	4.47	0.265	0.187	90.21
MESF 200 mg	3.67	4.86	2.37	3.63	0.587	0.415	54.46
MESF 400 mg	3.87	3.12	5.09	4.02	0.468	0.33	71.06

In the table M-1 = mice 1, M-2 = mice 2, M-3 = mice 3
The data in Table: 11.6 showed that after 90 minutes the central analgesic action of Crude Methanol extract at a dose of 400 mg/kg became stronger. It showed a percent elongation of 71.06%. At a dose of 200 mg/kg showed a percent elongation of 54.46%.

Table 10.7: Statistical Evaluation of the data obtained after 90 minutes

Group	t-Test value	Degree of freedom	P value	Level of significance
Standard (Diclofenac)	29.2161	2	.000585	Very statistically significant
MESF (200 mg)	10.7110	2	.004302	Very statistically significant
MESF (400 mg)	14.8779	2	.002244	Very statistically significant

The statistical evaluation of the data showed that the central analgesic activity of the samples declined after 90 minutes.

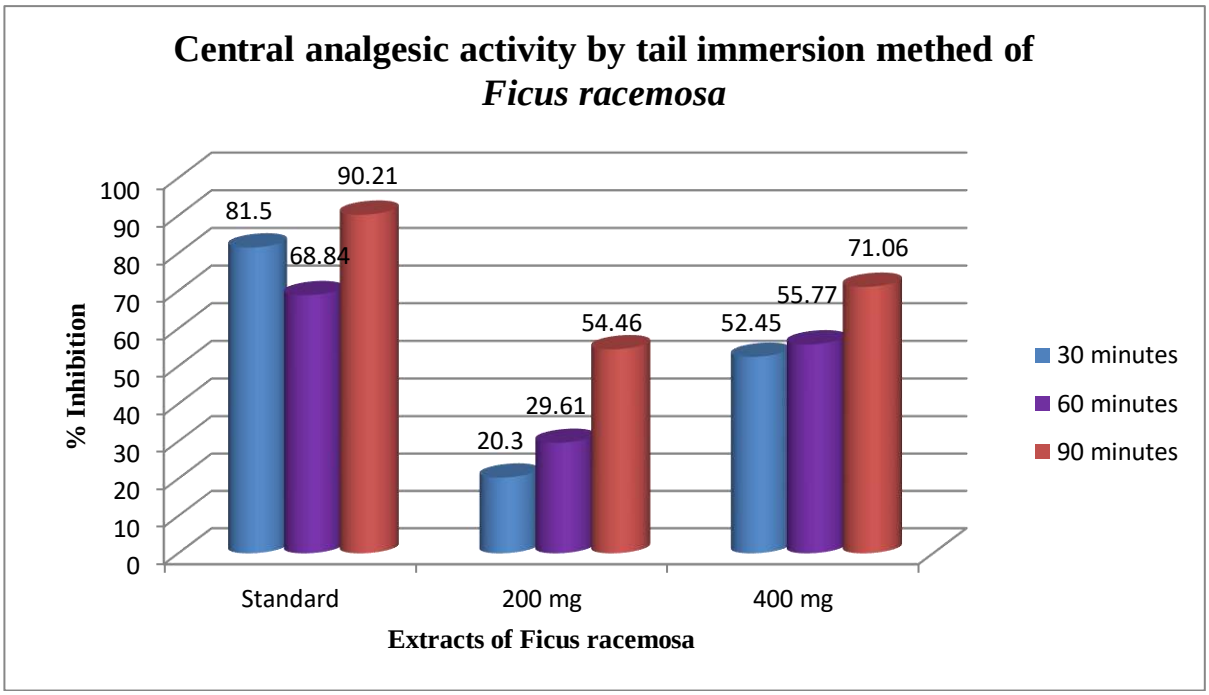


Figure 10.3: Comparison of percent time elongation of tail immersion of different samples (Author's own work)

The analysis and statistical evaluation of the data leads to the following important conclusions.

- The crude methanol extract has significant central analgesic activity at 400 mg/kg dose.
- The central analgesic activity is highest after 90 minutes.
- As the time progress the analgesic activity decreases. After 90 minutes there is almost no central analgesic activity in the plant extracts.

Evaluation of Hypoglycemic Activity:

11.1 Principle

Diabetes mellitus is the most common endocrine disorder in men and women and the major public health problem of epidemic proportion. Once believed to be a disease of west, it is becoming an endemic to modernizing and urbanizing population in our country. Ayurvedic literature reveals that many herbal medicines in different oral formulation have been shown to comprise potent hypoglycemic activity and therefore recommended in madhumeha (diabetes mellitus) and confident claims of cure are on record.

A glucose tolerance test (GTT) is one of the most acceptable methods to evaluate the hypoglycemic activity. It is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism. Many variations of the GTT have been devised over the years for various purposes, with different standard doses of glucose, different routes of administration, different intervals and durations of sampling, and various substances measured in addition to blood glucose.

At the present study, hypoglycemic effect of methanolic extract of roots of *Ficusracemosa* at 250 mg/kg and 500 mg/kg doses were examined & compared with relative to that of control and standard group. Here Glibenclamide was used as a standard drug.

11.2 Experimental Animal

Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B) were used for the experiment. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 h light-dark cycle and fed ICDDR; B formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Figure 11.1: Swiss albino mice

Figure 11.2: Oral administration (Author's own work)

11.3 Experimental Design

Twelve experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III (A), and group- III (B) consisting of 3 mice in each group. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. As it was difficult to observe the biologic response of three mice at a time receiving same treatment, it was necessary to identify individual animal of a group during the treatment. The

animals were individualized in the following way (Figure 13.3) and marked as M-1=Mice 1, M-2=Mice 2, and M-3=Mice 3.

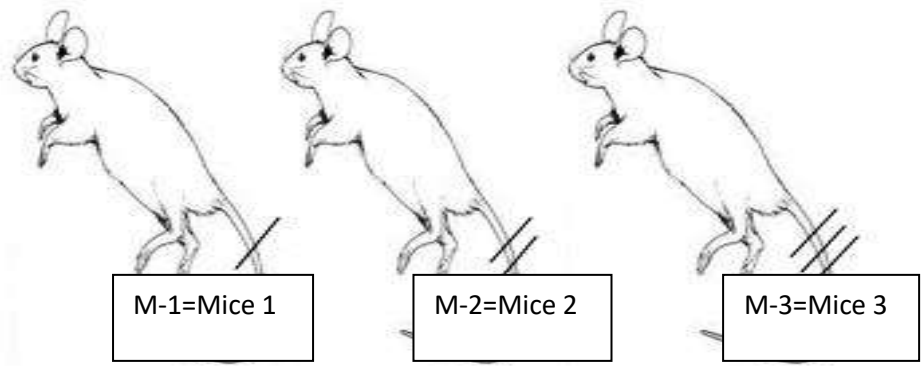


Figure 10.3: Numbering of mice (Author's own work)

11.3.1 Preparation of Test Materials

In order to administer the extract at doses of 400 mg/kg body wt and 200 mg/kg body wt of mice, the exactly weighed extracts were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture.
For the preparation of standard (Glibenclamide) at the dose of 10-mg/kg body weight, 10 mg tablet was dissolved into 3.0 ml distilled water.

11.3.2 Procedure

At zero hour, blood glucose level of each animal of each group was determined by glucometer and 0.6 ml 10% glucose solution (2 gm/ kg body wt) was administered orally by means of a long needle with a ball shaped end.

⇩

After 25 minutes, extract of two concentrations was administration into the test group, Glibenclamide solution into positive control group and water was administration into negative control group.

⇩

After 60, 120 & 180 min of glucose loading, blood samples were collected from tail vein.

⇩

By using glucometer blood glucose level was measured

Figure 11.4: Schematic representation for determining blood glucose root of mice after administration of *Ficusracemosa* extract.

Figure 11.5: Pricking of mice's tail (Author's own work)

11.4 Result & Discussion of hypoglycemic activity of test materials of *Ficusracemosa.*

The effects of methanolic extract of stem roots of *Ficusracemosa* at 200 and 400 mg/kg dose and its different fractionates at 200 mg/kg doses to lower blood glucose level were observed as follows to evaluate their hypoglycemic activity-

Table 11.1: Test materials used in the evaluation of hypoglycemic activity of crude extract and its different fractions of *Ficusracemosa.*

Code no.	Test Samples	Group	Identification	Dose (mg/kg)*
CTL	1% Tween-80 & DMSO in normal saline	I	Control Group	0.1 ml/10 g of body wt
STD	Glibenclamide	II	Standard Group	10
MESF 200mg	Methanolic extract of *Ficusracemosa.*	III A	Test Sample	200
MESF 400mg	Methanolic extract of *Ficusracemosa.*	III B	Test Sample	400

Table 11.2: Plasma level of glucose (mmol/L) of mice at different times.

Group		0 Min		Glucose+ 25 min		24 Hours		48 Hours		72 Hours	
		Data	Mean	Data	Mean	Data	Mean	Data	Mean	**Data**	Mean
Control (H_2O)	M-1	8.3	6.86	11.4	9.8	7.8	7.8	7.9	7.83	6.4	6.2
	M-2	5.3		9.8		8.9		7.8		6.0	
	M-3	7.0		8.2		6.7		7.8		6.2	
Standard	M-1	7.1	6.56	8.9	10.9	8.7	6.63	8.2	7	6.5	6.36
	M-2	6.7		14.4		5.3		5.8		6.2	
	M-3	5.9		9.5		5.9		7.1		6.4	
200 mg	M-1	8.3	8.6	11	11.3	6.7	6.7	5.5	7	6.3	6.4
	M-2	8.6		11.6		7		7.2		6.4	
	M-3	8.9		113		6.4		8.3		6.5	
400 mg	M-1	10.4	10.43	13.3	13.8	8.8	7.73	10.5	8.7	7.8	7.93
	M-2	10.9		16.2		7		6.9		7.7	
	M-3	10		11.9		7.4		8.7		8.3	

Table 11.3: Hypoglycemic activity of crude extract and its different fractions of *Ficusracemosa.*

Code	Plasma level of glucose (Mean)					Mean	SD	SE
	0 min	Glucose+ 25 min	24 Hour	48 Hours	72 Hours			
Control	6.86	9.8	7.8	7.83	6.2	7.8	0.545	0.2725
Standard	6.56	10.9	6.63	7	6.36	7.49	0.7679	0.383
200 mg	8.6	11.3	6.7	7	6.4	8	0.812	0.406
400 mg	10.43	13.8	7.73	8.7	7.93	9.71	1.006	0.503

Table 11.4: Statistical evaluation of the Data

Code	SE	t Test value	Degree of freedom	P value	Level of significance
Control		-	4		-
Standard		21.8103	4	.000013	Extremely statistically significant
200 mg		22.0302	4	.000013	Extremely statistically significant
400 mg		21.5827	4	.000014	Extremely statistically significant

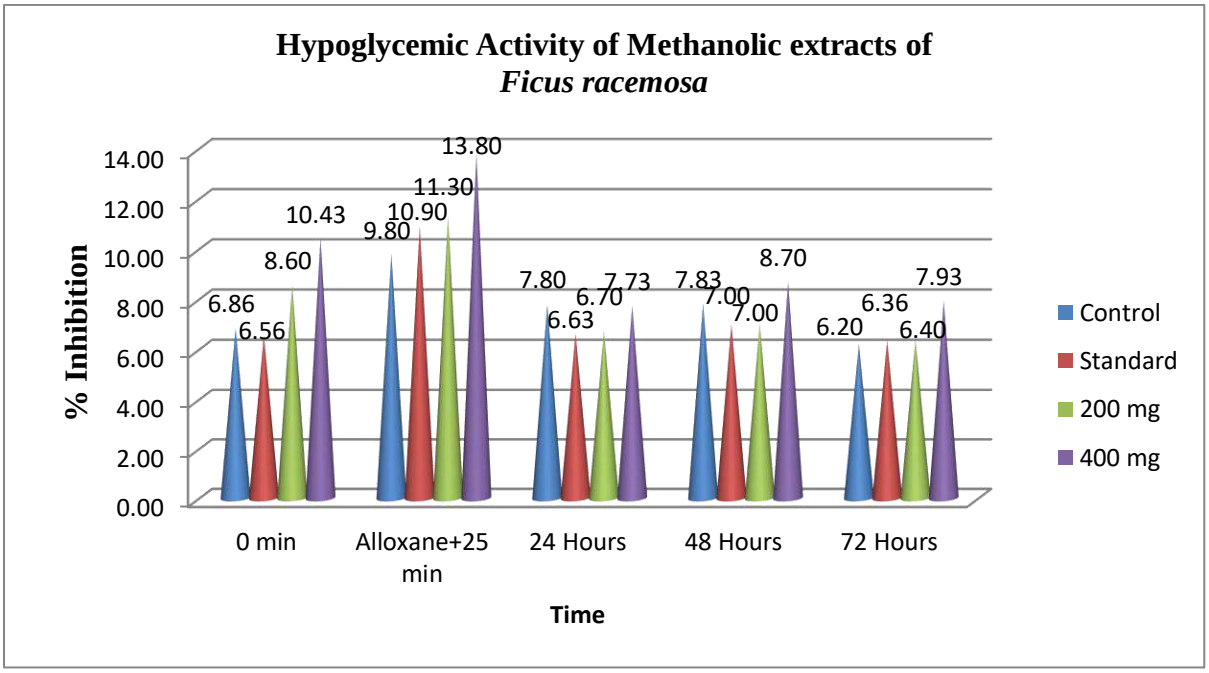


Fig 11.6: Plasma level of glucose of different groups of mice at different times (Author's own work)

The methanolic extrat of roots of *Ficusracemosa* has statistically significant blood glucose lowing activity at dose of 400 mg/kg. But significant hypoglycemic activity was exhibited by any other test subject. So, it can be concluded that the crude methanolic extract possesses moderate hypoglycemic activity at 400 mg.

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