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Medicinal and Biological Investigations of *Ficus racemosa*

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Key words: *Ficus racemosa*, phytochemical screening, thrombolytic activity, antimicrobial activity, medicinal plants of Bangladesh

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Abstract: *Ficus racemosa* is a traditional medicinal plant found in Southeast Asia, India, Australia. It is commonly known as 'gular'or 'Dumur'. Owing to the presence of β-sitosterol it decreases the blood glucose concentration. Many active constituents isolated from different parts of this plant exhibit useful pharmacological activity. 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. Some chemical and biological researches on this plant have been conducted out, so far, focusing mainly on the plant's bark and root. That's why the objective of this framework is to assess the different possibilities of developing new therapeutic targets from this plant's fruit that could be crucial for the treatment of many diseases.

INTRODUCTION

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] (Fig. 1).

Written records of plant use as therapeutic agents date back millennia. The ancient records are from Mesopotamia and date back to around 2600 BC. These records are not just a case of one or two plant-based 'drugs' finding their way into common use as the documents indicate that plants contained many drugs in use (up to 1,000 in the particular instance of Mesopotamia)[2]. Around 119 pure chemical substances extracted from the higher plants are used worldwide in medicine. In most medicinal herbs, fresh or dried parts whole chopped, powdered or an advanced form of herb, usually produced by a solvent such as water, ethanol or organic solvent, play an important role and are the backbone of traditional medicines[3].

Fig. 1: *Ficus racemosa* tree

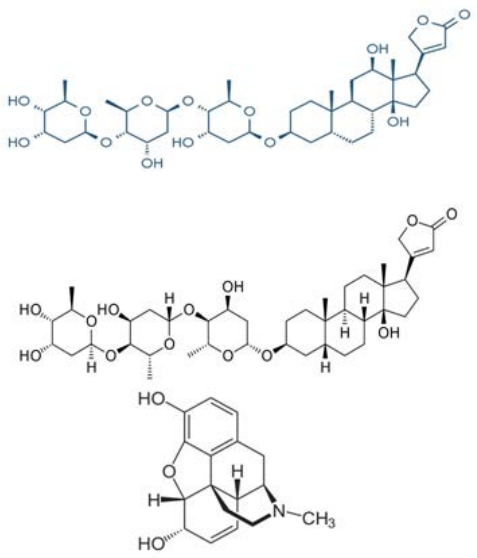

Fig. 2(a-c): Lead compound search and utilization from plants (a) Digoxin (Chemistry World) (b) Digitoxin (Science direct.com) and (c) Morphin (ChemSpider)

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.

To generate bioactive compounds of novel or known structures as semi-synthesized lead compounds to produce protectable entities with higher activity and/or lower toxic effects, e.g., metformin, nabilone, oxycodone, taxotere, teniposide, verapamil and miodarone based on galegin, A9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin, khellin. 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 s(c) (Fig. 2).

Unmodified natural products of which the therapeutic efficacy was only remotely suggested by folk medicine use e.g., vincristine. Modified natural or synthetic substances based on a natural product used in traditional medicine, e.g., aspirin[5].

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].

A careful selection of plants is conditioned by success in the research of natural products, based on various criteria such as chemotaxonomic data, ethnomedical information, field observations or even discrete collection[8]. Historical encounters with plants as therapeutic instruments helped to introduce particular chemical entities into 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 development from terrestrial plants has relied mainly on bioactivity-guided isolation methods which haveled, for example, to the discovery of essential anticancer agents, Taxus brevifolia paclitaxel and Camptotheca acuminate camptothecin[6]. The 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, vinbiastine, vincristine 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, D9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin and khellin; to use agents as pharmacologic tools, e.g., Lysergic acid Diethlamide (LSD), mescaline, yohimbine and to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, etc. The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000[9] with a lower level at 215,000 and an upper level as high as 500,000[10]. Of these, only about 6% have been screened for biologic activity and a reported 5% have been evaluated phytochemically[11]. It was estimated that in 1991 in the United States, for every 10,000 pure compounds most likely those based on synthesis) that are biologically evaluated (primarily *in vitro*), 20 would be tested in animal models and 10 of these would be clinically evaluated and only one would

reach US Food and Drug administration approval for marketing. The time required for this process was estimated at 10 years at a cost of $231 million (US)[12]. 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[13]. Chemical diversity of secondary plant metabolites that results from plant evolution is superior to at found in synthetic combinatorial chemical libraries[12]. 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 plant[14]. The medicinal plants are used in the pharmaceutical, cosmetic, agricultural and food industries. With the onset of scientific research into herbals, it becomes increasingly clear that medicinal herbs have potential in today's synthetic era as numbers of medicines become 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 to identify alternative and complementary medicine. To reduce the toxicity of drug therapy especially toxicity reduction of synthetic and semi synthetic drugs. To find the lead compound diversification to treat various diseases[17].

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[16]. 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.[16]. Treatments in traditional medicine involve internal and external use of medicinal products, physical manipulation of different parts of the body, performing rituals, psychological treatment and also minor surgery. Ayurvedic medicinal preparations consist mainly of powdered plant materials, semi-solid preparations, decoctions, elixirs and distillate. Many of these also contain inorganic chemical substances, minerals and products for animals. Ayurvedic medicine also uses alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs[18]. The major constituents of Unani medicine are whole plants or their powders or pastes or products and their extracts, infusions, decoctions and distillates. In the preparation of these medicines, minerals, inorganic chemicals and animal products are also frequently used. The Indian subcontinent's medical knowledge is termed Ayurveda for hundreds of years. 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[17]. Some crude drugs used as medicine in Bangladesh are reported in Table 1.

Research of traditional drugs in Bangladesh: Medicinal plants have long been used in rural areas as a remedy for illness. Not only do they cure the disease but they also play an important part in the economy. Medicinal plants are cheap and easy to reach people who have known this very well. Bioactive molecules are deposited in medicinal plants they can serve as important raw materials for the production of pharmaceutical products. They comprise a

Table 1: Some crude drugs used as medicine Bangladesh[18]

Plants	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 and cold, chronic bronchitis and asthma, expectorant
Anantamul	*Hemidesmus indicus*	Root	Essential Oil, Saponin, Resin, Tannins, Sterols and glucosides	Tonic, diuretic, demulcent, diaphoretic, carminative
Arjun	*Tarminalia arjuna*	Leaves and bark	Tannins, ß-sitosterols, saponin	As cardio tonic in angina pain, diuretic in palpitations
Chirata	*Gentiana chirayita*	Entire dried plant	Gentiopicrin (bitter glycoside)	Bitter tonic, febrifuge, stomachic and 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 and 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 and 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 and spasmolytic
Neem	*Azadiachta indica*	Leaves and seed oil	Nimbin, nimbinene, nimbandiol (indole alkaloids)	Stimulant, antiseptic used in rheumatism and 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 B12	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

country's valuable asset and donate to its health-care system. Well-judged and scientific research into this wealth can contribute significantly to the public health. Besides being commodities available for trade. A country can also earn a good amount of foreign currency by exporting this natural wealth to other countries. About <500 of such medicinal plants have so far been established as growing in Bangladesh[18]. 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 various villages in Bangladesh shows that an average of 14% of people suffering from disease approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mullahs, 29% contact quack and 19% contact homeopaths. The survey shows an extensive use by our people of medicinal plants, the majority of which are served in crude and inferior form[19]. Traditional medicines are still manufactured in our country by following the age-old unscientific, traditional methods. Hundreds of traditional medicinal plants are employed in various Ayurvedic and Unani commercial preparations without proper standardization, quality control, assessment and perseverance of the chemical nature, pharmacological and toxicological studies of the active ingredients which are essential for the full utilization of their medicinal properties. 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[18]. 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.

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[20]. The 'Susruta samhita' and the 'charaka samhita' were influential works about traditional medicine during the Vedic period. Hundreds of medicinal plants have been identified and have traditionally been in use since. Ayurvedic practitioners have developed a number of medicinal preparations and surgical procedures for the treatment of various diseases and disorders over the following centuries. WHO estimates that 80% of populaces living in developing countries rely on traditional medication exclusively for their primary health

care needs. Traditional plants play a major role in almost all herbal medicine and are the backbone of traditional medicine[21]. 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 b-adrenoceptor antagonists and cimetidine and other H_2receptor 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 led to high throughput screening[20]. 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 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[20]. 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[22].

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[23]. 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

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:

- Selection and correct identification of the proper medicinal plants and its extraction with a suitable solvent
- 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

- Fraction of the crude extracts by using physico-chemical procedure and monitored by biological tests identification and separation of the active fractions
- Isolation of the active constituents by chromatographic or other technique and purification of the isolation compounds by repeated chromatography and crystallization
- Establishment of the chemical structures of the pure compound by various physicochemical techniques and determination of their biological activity by various pharmacological tests

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:

- Novel structure of biologically active chemical compounds isolated from plants sources, often to prompt the chemists to synthesis similar or better semi-synthetic compounds
- Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant derived compound with known biological activity
- 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, methyldihy dromorphinone, 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.

Status of medicinal plants of Bangladesh:

- About 500 medicinal plants have been reported to occur in Bangladesh
- Almost 80% of rural populations are dependent on medicinal plants for their primary health care
- The local people conserve traditional knowledge through their experience and practices which is handed down orally without any documentation
- The over exploitation of wild medicinal plants has become a threat to its extinction
- In Bangladesh there is no systematic cultivation process of conservation strategies about medicinal plants
- There is no government policy or rules and regulations about the medicinal plants cultivation conservation and marketing
- There are almost 422 herbal medicinal companies using medicinal plants as raw materials mostly by importing from abroad

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:

- Volatile or essential oils
- Fixed oils
- Gum-resins and mucilage
- Alkaloid and amines
- Pyridine group
- Tropane group
- Isoquiloline group
- Quinoline group
- Quinolizidile group
- Indole group
- Steroidal group
- Phenylethylamine group
- Alkaloid amines

Glycosides:

- Anthraquinone glycoside
- Cardiac glycoside
- Saponin glycoside Thiocyanate glycoside
- Other glycoside
- Vitamin and mineral

Flow chart of bioactivity guided phytochemical approach: Figure 3 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.

PLANT PREVIEW

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

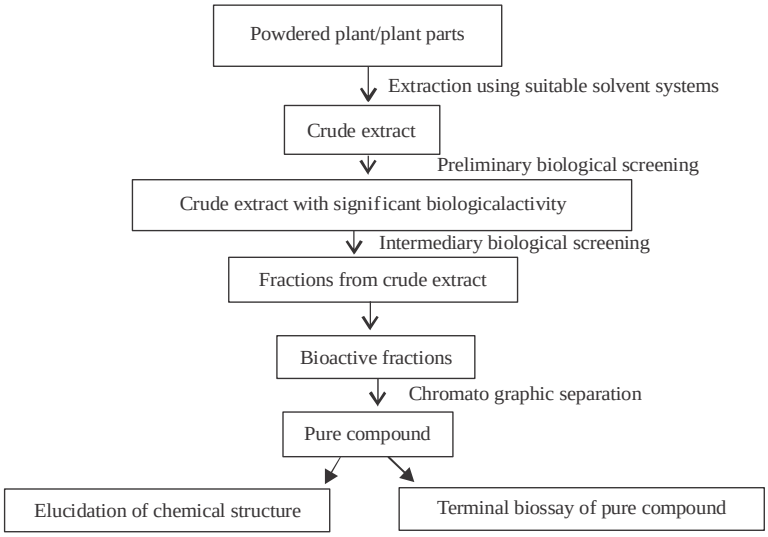

Fig. 3: Flow chart

distinctive aspect of this tree is the red, furry figs in short clusters which grow directly out of the trunk of the tree[24].

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 *Ficus racemosa* Linn is 3/4 inch to 2 inches long, circular and grows directly on the trunk[25].

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

Fig. 4: *Ficus racemosa* plant (Tropical Ferns)

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[26].

Roots: The roots of *F.racemosa* are long, brownish in color. It's having characteristic odor and slightly bitter in taste Roots are irregular in shape (Fig. 4-6).

Vernacular names:

- Scientific Name: *Ficus racemosa*
- Synonyms: *Ficus racemosa* var. *mollis*, *Ficusglomerata*
- Bengali: Dumor, 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

Fig. 5: The flowers of *Ficus racemosa* (Ayushvedah)

Fig. 6: The flowers of *Ficus racemosa* (Ayushvedah)

- 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

Recent taxonomic revisions of the family include these genera[27]:

- *Ficus chittagonga*
- *Ficus glomerata*
- *Ficus glomerata var. chittagonga*
- *Ficus glomerata var. elongata*
- *Ficus glomerata var. miquelii*
- *Ficus glomerata var. mollis*
- *Ficus goolereea*
- *Ficushenrici*
- *Ficuslanceolata*
- *Ficuslucescens*
- *Ficusmollis*
- *Ficus racemosa var. miquelii*
- *Ficus racemosa var. mollis*
- *Ficus racemosa var. vesca (F. Müll. ex Miq.) Barrett*
- *Ficussemicostata*
- *Ficustrichocarpa*

Classification (*Ficus racemosa* 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: *Ficus racemosa*

Plant description: Ficusracemosa, 20-30 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[28]. Leaves simple, alternate, stipules 12-18 mm long, lanceolate, linear lanceolate, pubescent, often persistent on young shoots; petiole 10-50 mm long, slender, grooved above, becoming brown scurfy; lamina 6-15×3.5-6 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, ovate, triangular, obtuse, persistent; flowers of unisexual, 4 kinds; male flowers near the mouth of receptacles, in 2-3 rings, sessile, much compressed; tepals 3-4, dentate-lacerate, lobes jointed below, red, glabrous; stamens 2, exserted; filaments 1 mm, connate below; anthers oblong, parallel; female flowers sessile or very shortly stalked among gall flowers; tepals 3-4, dentate-lacerate, lobes jointed below, red, glabrous, ovary superior, sessile or substipitate, red spotted; style 2-3 mm long, glabrous, simple; stigma clavate; gall flowers long stalked; ovary dark red, rough; style short. Syconium 2.×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: *Ficus racemosa* 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 *Ficus racemosa.* 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 m 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.

Chemistry of the plant *Ficus racemosa:* 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[29].

Medicinal uses of the plant *Ficus racemosa:*

- The leaves are used in the treatment of diarrhea
- The bark is astringent. It is used in the treatment of hematuria, menorrhagia and hemoptysis
- The fruit is astringent. It is used in the treatment of hematuria, menorrhagia and hemoptysis. 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 gonorrhea
- The root is chewed as a treatment for tonsillitis

Medicinal Applications of the plant *Ficus racemosa:* A few of the health benefits derived from figs include-Prevention of constipation: There are 5 g 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 *Ficus racemosa* 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: *Ficus racemosa* 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. *Ficus racemosa* 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 *Ficus racemosa* 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, *Ficus racemosa* 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: *Ficus racemosa* 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.

Table 2: Experimental plant

Name of plant	Family	Plant part
Ficus racemosa	Moraceae	Flower

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.

Pharmacological activities of *Ficus racemosa*

Antimicrobial activity: Methanolic extracts of *Ficus racemosa* 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^{-1}). In both the cases, the methanolic extracts at dose concentration of 500 µg mL^{-1} showed significant antimicrobial activity.

Analgesic activity: Saponin (SN) isolated from *Ficus racemosa* 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^{-1} 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[30].

Antibacterial activity: The methanol extract of flowers of the plant have been screened for their antibacterial activity. The extract (7.5 mg $disc^{-1}$) showed broad-spectrum antibacterial activity against gram positive and gram negative bacteria. The results were compared with the standard drug streptomycin (10 µg $disc^{-1}$). The zone inhibition was found to be increased with the increase in concentration of the extract and thus exhibiting concentration dependent activity[31] (Table 2).

PREPARATION OF PLANT EXTRACT

Biological investigation of the experimental plants:

- The investigations of the plant will be discussed in two different sections
- Phytochemical investigation
- Biological investigation

Biological investigation of *Ficus racemosa*

Collection and preparation of the plant material: The whole plant of *Ficus racemose* was collected from Khamarbari, Farmgate, Dhaka and identified by taxonomist of National Herbarium, Bangladesh situated

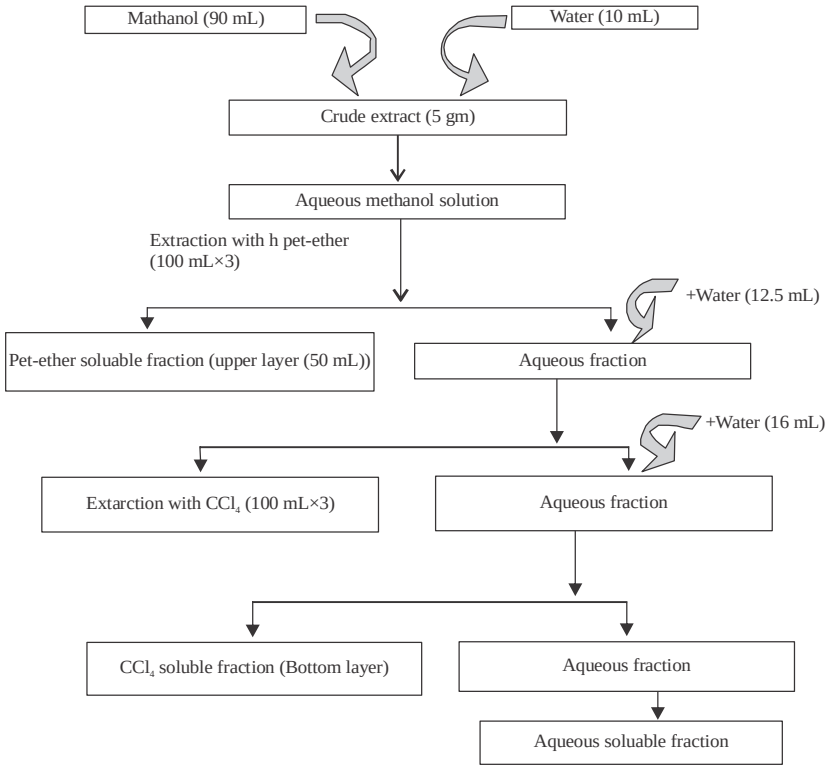

Fig. 7: Schematic representation of the modified Kupchan partitioning of crude extract of root of *Ficus racemosa*

at Mirpur in Dhaka. The sample is preserved in the Phytochemical Laboratory of World University of Bangladesh for as further reference (Accession No. 41878). For several days, the fruit air dried and then the oven dried for 24 h at considerably low temperatures (not >400°C) for better grinding. In the Phytochemical Research Laboratory, Faculty of Pharmacy, World University of Bangladesh (WUB), the dried fruits were then ground to a coarse powder using high-capacity grinding machine.

Extraction of the plant material: The powdered material (250 g) was taken in a cleaned, amber colored reagent bottle (5 L) 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.

Solvent-solvent partition of crude extract by modified Kupchan partition method: Solvent-solvent partitioning was done using the protocol designed by Van Wagenen *et al*.[32]. The crude extract (5 g) 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 Fig. 7. All the five fractions were evaporated to dryness (Table 1) and were used for further analysis.

Partitioning with Petroleum ether: The mother solution was taken in a separating funnel. About 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.

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×3). The carbon tetrachloride fractions were collected together and evaporated. The aqueous fraction was preserved for the next step.

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

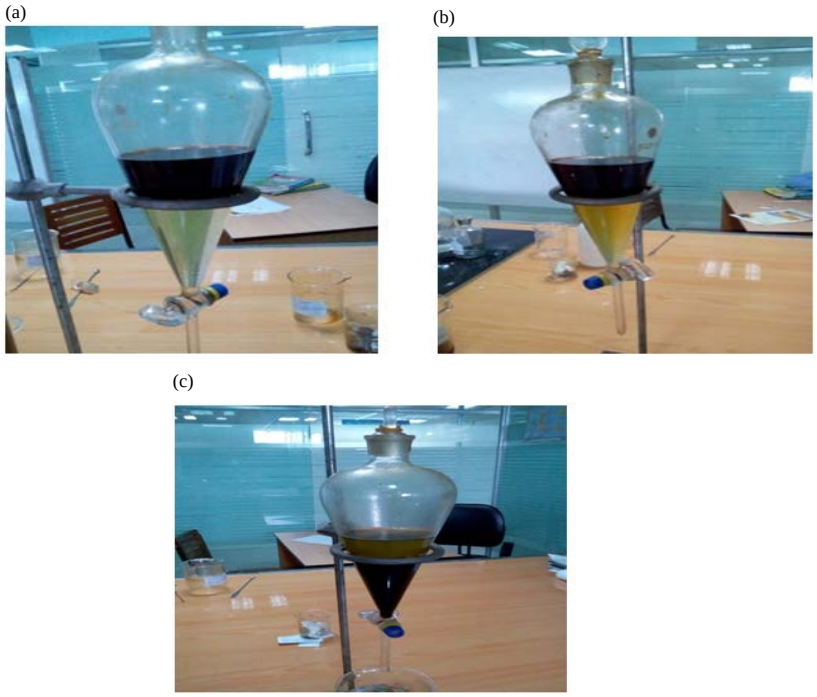

Fig. 8(a-c): Partitioning of plant extract (a) CHCl3 Soluble Fraction (b) Carbon Tetra Chloride soluble fraction and (c) Petroleum ether soluble fraction

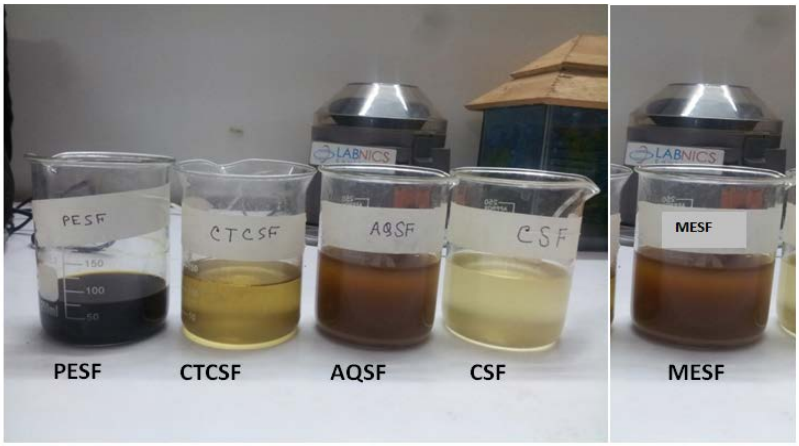

Fig. 9: Different fractions of *Ficus racemosa* (Author's own work)

Table 3: Fractions obtained from *Ficus racemosa* extract

Plant part	Sample code	Fraction
Flowers of *Ficus racemosa*	MESF	Methanol Extract
	PESF	Pet-ether Soluble Fraction
	CTCSF	Carbon Tetrachloride Soluble fraction
	CSF	Chloroform Soluble Fraction
	AQSF	Aqueous Sluble Fraction

mixed uniformly. The mother solution was then taken in a separating funnel and extracted with Chloroform ($CHCl_3$) (100 mL×3). The $CHCl_3$ soluble fractions were collected together and evaporated. The aqueous methanol fraction was preserved as aqueous fraction (Fig. 7-9 and Table 3).

DESIGN OF BIOLOGICAL INVESTIGATION

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. Of the 520 new drugs accepted between 1983 and 1994, 39% were organic or derived from natural products and 60-80%t were derived from natural products. Thirteen drugs related to natural products were approved between 2005 and 2007 and five of these represented the first members of new drug classes: peptides exenatide and ziconotide and the small molecules ixabepilone, retapamulin.

There is also current commercial evidence supporting the case for natural products. Of the 20 best-selling non-protein drugs in 1999, nine were eitherderived from or developed as a result of naturally occurring leads 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[33].

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.

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 2.

Results and discussion: Phytochemical studies showed that Alkaloids, Steroids, Flavonoids, Tannins, Saponin, Gum and 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

Microbiological investigations: With the help of an in vitro antimicrobial study, the antibacterial as well as antifungal spectrum of the crude extracts can be determined by observing the growth response. These studies are based on the fact that many infections are caused by bacteria and pillows and if the test materials inhibit bacterial or fungal growth, they can be used in these infections. However, a number of factors can influence the results like:

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

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.

Membrane stabilizing activity investigation: Inflammatory cells produce a complex mixture of growth differentiation cytokines as well as physiologically active arachidonate metabolites. They also have the ability to generate Reactive Oxygen Species (ROS) that can damage cellular biomolecules, thus increasing the inflammatory state[34]. Erythrocyte membrane is similar to lysosomal membrane and, for example , the effect of drugs on erythrocyte stabilization can be extrapolated to the stabilization of lysosomal membrane[35]. Therefore, when the membrane stabilizes, it interferes with the release and action of mediators such as histamine, serotonin, prostaglandin, leukotrins, etc.[36].

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

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^{-1}) but symptoms may not start to become noticeable until even higher values such as 250-300 mg dL^{-1}. A subject with a consistent range above 126 mg dL^{-1} is generally held to have hyperglycemia. Chronic levels exceeding 125 mg dL^{-1} can produce organ damage. 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. About >400 species have been reported to display hypoglycemic effects but only few of them have been investigated[37] and the World Health Organization has recommended that this area warrants attention[38].This study was thus undertaken to evaluate the hypoglycemic effect of a methanolic extracts of *Ficus racemosa* flowers and their different fractionates.

Anti-diarrheal activity investigation: Diarrhea is characterized by an increase in the frequency of bowel movements, wet stools and abdominal pain. It is the world's third-largest killer disease, contributing significantly to pediatric morbidity and mortality, especially in the undernourished. 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)

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[39]. 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[40]. 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[41]. 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[42]. 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, tototrienols, 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-Butyl HydroQuinone (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 because of the possibility that they might underlie the protective effects afforded by fruit and vegetable intake against cancer and other chronic diseases. Due to the complex nature of phytochemicals, the antioxidant activity of plant extracts must be assessed by combining two or more different. *In-vitro* assays. A number of publications on the isolation and testing of plant-derived antioxidants have been characterized over the last decade. The purpose of this study was to evaluate

different extractives of roots of *Ficus racemosa* as new potential sources of natural antioxidants and phenolic compounds.

Assays for total phenolic content: The antioxidant effect is mainly due to phenolic compounds such as flavonoids, phenolic acids and phenolic diterpenes. Phenolic compounds exert their antioxidant properties by redox reactions which may play an important role in the absorption and neutralization of free radicals, the quenching of singlet and triplet oxygen or the decomposition of peroxides. Many phytochemicals have strong antioxidant capabilities that may be associated with lower incidence and lower rates of cancer mortality in a number of human populations. Phenolic compounds are secondary metabolites of plants and can act as antioxidants through many potential pathways such as radical scavenging, oxygen radical absorption and chelating of metal ions[43].

Principle: Phenols ionize completely in the alkaline state. When the Folin-Ciocalteu reagent is used in this ionized phenolic solution, the reagent will easily oxidize the phenols. The usual color of the Folin-Ciocalteu reagent is yellow and the solution becomes blue after the oxidation process. The intensity of the color change is measured at 760 nm in the spectrophotometer. The absorbance value will reflect the total phenolic content of the compound[44] (Fig. 10).

Materials and methods: Total phenolic content of root of Phytochemical and biological investigations of *Ficus racemosa* extractives was measured employing the method as described earlier[45] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard[46].

Materials:

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

Composition of folin-ciocalteu reagent[47] (Table 4)

Standard curve preparation: Gallic acid was used here as standard. Different gallic acid solutions were prepared having a concentration ranging from 100 to 0 µg mL^{-1}. 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 min at room temperature. After 20 min 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.

Sample preparation: About 2 mg of the extractives was taken and dissolved in the distilled water to get a sample concentration of 2 mg mL^{-1} in every case. The samples along with their concentration for the total phenolic content measurement are given in Table 5 and Fig. 11.

Total phenolic content analysis: To 0.5 mL of extract solution (conc. 2 mg mL^{-1}), 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 min at room temperature. After 20 min 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)/g of the extract[48].

Results and discussion of the test samples of fruits of Ficusracemosa

Total Phenolic Content (TPC): The Methanolic Extract of *Ficus racemosa* (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 6) equivalents, results of the colorimetric analysis of the total phenolics are given in Table 7). Total phenolic content of the samples are expressed as mg of GAE (Gallic Acid Equivalent)/g of extractives.

The amount of total phenolic content differed in different extractives and ranged from 4 mg of GAE/g of extractives to 25.43 mg of GAE/g of extractives of *Ficus racemosa* among all extractives of *Ficus racemosa* the highest phenolic content was found in CTCSF (77.63 mg of GAE/g of extractives) followed by AQSF

Table 4: Results of different chemical group test of the Methanol extract of *Ficus racemosa*

Samples	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 and 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 min	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 min 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

Table 5: Composition of Folin-Ciocalteu

Components	Percentage
Water	57.5
Lithium Sulfate	15.0
Sodium Tungstate Dihydrate	10.0
Hydrochloric Acid = 25%	10.0
Phosphoric Acid 85% solution in water	5.0
Molybdic Acid Sodium Dihydrate	2.5

Table 6: Test sample for total phenolic content determination

Plant part	Sample code	Test sample	Calculated amount (mg mL^{-1})
Flowers of *Ficus racemosa*	MESF	Methanol extract of fruits of *Ficus racemosa*	2.0
	PESF	Pet-ether soluble fraction	2.0
	CTCSF	Carbon tetrachloride soluble fraction	2.0
	AQSF	Aqueous soluble fraction	2.0

Table 7: Standard curve preparation by using gallic acid

x = GA Conc. (μg mL^{-1})	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	0.020		
0.000	0.011		

(49.44 mg of GAE/g of extractives).Significant amount of phenolic compounds were also present in MESF (56.13 mg of GAE/g of extractives), PESF (51.19 mg of GAE/g of extractives) are also found (Fig. 12 and 13).

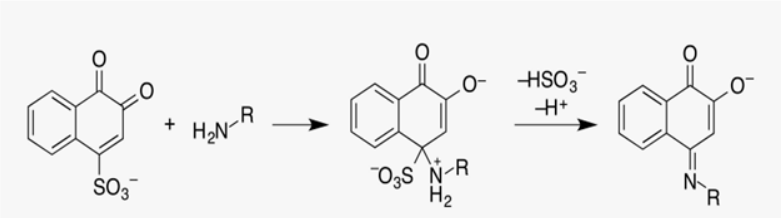

Fig. 10: Folin Ciocalteu reagent reaction with phenol (PNG items)

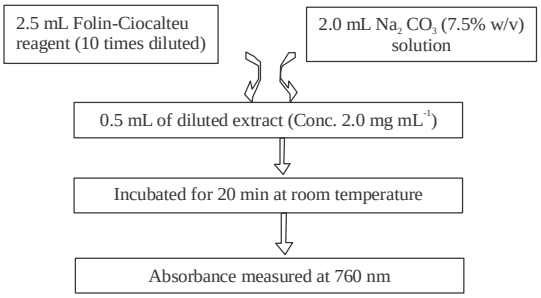

Fig. 11: Schematic representation of the total phenolic content determination

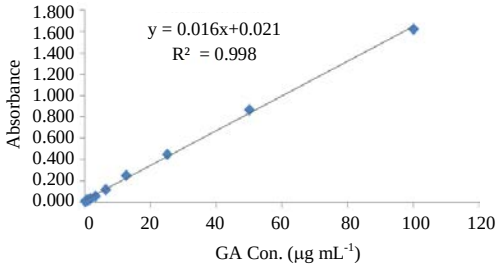

Fig. 12: Standard curve of gallic acid for total phenolic content determination (Researcher's own work)

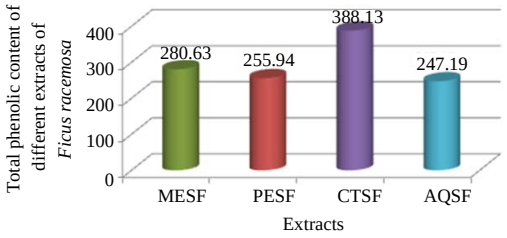

Fig. 13: Total phenolic content (mg of GAE/gm of extractives) of different extractives of leaf of *Ficus racemosa* (Resaercher's own work)

ANTIMICROBIAL ACTIVITY

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[49]. 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[49].

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[50]. 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[51]. Disc diffusion method (Table 8):

- Serial dilution method
- Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening[52]. 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[53]. Among the above mentioned techniques the disc diffusionis a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity[53]. It is essentially a quantitative or qualitative test indicating the

Table 8: Test samples for Total Phenolic Content (TPC) determination

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

sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method[54].

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 h to allow maximum diffusion of the test materials to the surrounding media[55]. The plates are then inverted and incubated at 37°C for 24 h 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[53, 55]. 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[53].

Experimental

Apparatus and reagents:

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

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 Table 9:

List of organization

Gram positive bacteria:

- *Bacillus subtilis*
- *Sarcina lutea*
- *Staphylococcus aureus*
- *Bacillus cereus*

Gram negative bacteria:

- *Escherichia coli*
- *Shigella dysenteriae*
- *Vibrio mimicus*
- *Vibrio parahemolyticus*
- *Salmonella typhi*

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.

Preparation of the medium: In order to prepare the required volume of this medium, the calculated amount of each of the components was taken in a conical flask and the distilled water was added to make the required volume. The contents were heated in a water bath to provide a clear solution. The pH (at 25°C) was adjusted to 7.2-7.6 using NaOH or HCl. The 10 and 5 mL of the medium were then transferred to the screw cap test tubes for the preparation of plates and slants. The test tubes were then capped and sterilized by 15-lbs autoclaving. Pressure at 121°C for about 20 min. Slants were used for the production of fresh culture of bacteria and fungi which were then used for the sensitivity study.

Sterilization procedure: Antimicrobial screening has been performed in Laminar Hood in order to prevent any type of contamination and cross-contamination by the test organisms and all types of precautions have been very well maintained. One hour before working in the Laminar Hood, UV light was switched on. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15 l bs sq^{-1}. About 20 min inch. Micropipette tips, cotton, forceps, blank disks, etc. were also sterilized by UV light[56].

Table 9: Preparation of sample discs

Plant part	Test sample	Dose µg disc^{-1}	Required amount for 20 disc (mg)
Flowers of *Ficus racemosa*	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

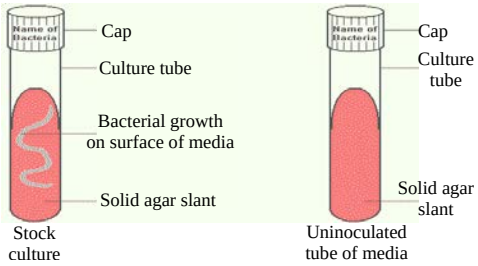

Fig. 14: Preparation of subculture (microbenotes.com)

Preparation of subculture: In a sterile conditions under a laminar air cabinet, the test organisms were transferred from pure cultures to agar slants with the help of a transfer loop in order to have fresh, pure cultures. The inoculated strains were then incubated for 24 h at 370°C for optimum growth. These fresh cultures have been used for the sensitivity test (Fig. 14).

Preparation of the test plate: The test organisms were relocated from the subculture to the test tubes containing approximately 10 mL of melted and sterilized agar with the help of a sterilized transfer loop in the aseptic area. The test tubes were shaken by rotation to obtain a uniform suspension of the microbes. The bacterial and fungal suspension was immediately transferred to the sterilized petridises. Petridis were rotated several times clockwise and clockwise to ensure the homogeneous distribution of the test organisms in the media (Fig. 15).

Preparation of discs: Measured amount of each test sample (specified in Table 7) 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 (Fig. 16 and Table 9).

Standard Ciprofloxacin (50 mg disc^{-1}) 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.

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°C for about 24 h upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 h.

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[57] (Fig. 17 and 18).

Results and discussion of *In vitro* antimicrobial screening of *Ficus racemosa:* The Methanol Extract of leaf of *Ficus racemosa* (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 mg disc^{-1} in every case. The results are given in Table 8.

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 7.0-9.0 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

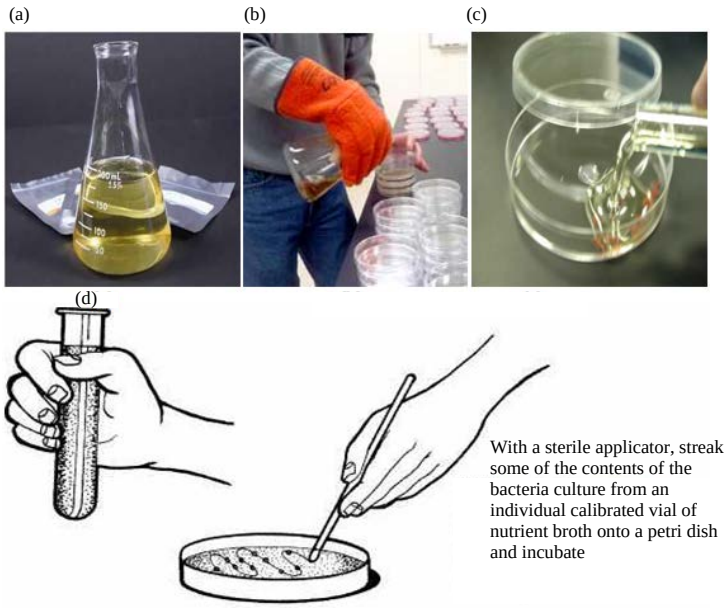

Fig. 15(a-d): (a) Preparation of the test plates (b) Freshly prepared culture medium, (c) Pouring culture medium to petridishes and (d) Transfer of bacterial and fungal suspension to the petridishes

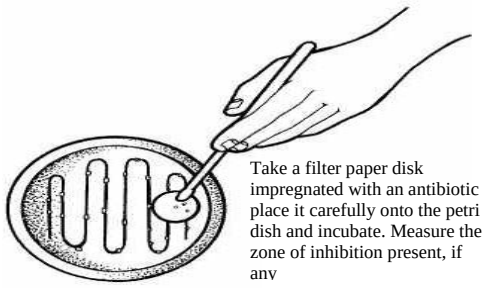

Fig. 16: Preparation of filter paper discs

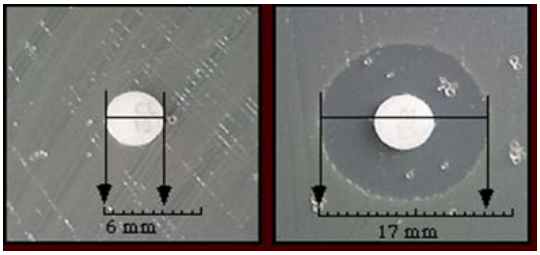

Fig. 18: Determination of clear zone of inhibition (Resaercher's own work)

Fig. 17: Clear zone of inhibition

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 7.0-9.0 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 7.0-8.0 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*

Table 10: Antimicrobial activity of test samples of *Ficus racemosa*

	Diameter of zone of inhibition (mm)					
Test M.O	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

Table 11: Preparation of different extracts of roots of *Ficus racemose*

Sample code	Concentration (mg mL^{-1})
Hypotonic solution	2
MESF	2
PESF	2
CTCSF	2
CSF	2
AQSF	2
ASA (Acetyl Salicylic Acid)	0.1

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 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 10).

MEMBRANE STABILIZING ACTIVITY

Introduction: Inflammation is one of the most important processes in many pathological disorders. Inflammatory cells produce a complex mixture of cytokine growth and differentiation as well as physiologically active arachidonate metabolites. In addition, they possess the ability to generate Reactive Oxygen Species (ROS) that can damage cell biomolecules which in turn increase the inflammation status[58]. Compounds that possess radical scavenging ability may, therefore, expect to have therapeutic potential for inflammatory disease[59]. Erythrocyte membrane is similar to lysosomal membrane and as such the effect of drugs on erythrocyte stabilization could be extrapolated to the stabilization of lysosomal membrane[35]. As a result, the membrane stabilizes that interfere with the release and/or action of mediators such as histamine, serotonin, prostaglandins, leukotrienes, etc.[60]. The aim of this study was to investigate the anti-inflammatory activity of the methanol extract and its different fractions of *Ficus racemosa* fruit (Table 11).

Materials and method

Preparation of the extract

Solvent used: Methanol analytical grade

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

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%.

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.

Phosphate buffer materials:

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

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 g monosodium phosphate dehydrate and 1.099 g 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%

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 0.16 M sodium chloride (NaCl) solution (approximately 0.95% salt in water) or 0.3 M 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 g NaCl was added and mixed.

Material for isotonic solution:
- Sodium chloride (NaCl)
- Water
- Glassware
- Stirring bar

Calculation for isotonic solution:
- About 1000 mL solution of strength 1 m contain = 58.5 g NaCl
- About 500 mL solution of strength 1 m contain = 58.5 2 g NaCl
- About 500 mL solution of strength 1000 mM contain = 58.5/2 g NaCl
- About 500 mL solution of strength 154 mM contain = 58.5×154/2×1000 g NaCl = 4.5045 g NaCl

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 g NaCl was added and mixed.

Materials for hypotonic solution:
- Sodium chloride (NaCl)
- Water
- Glassware
- Stirring bar

Calculation for hypotonic solution:
- About 1000 mL solution of strength 1 m contain = 58.5 g NaCl
- About 500 mL solution of strength 1 m contain = 58.5/2 g NaCl
- 500 mL solution of strength 1000 mM contain = 58.5/2 g NaCl
- About 500 mL solution of strength 50 mM contain = 58.5 × 50/2×1000 g NaCl
  = 1.4625 gm NaCl

Effect on hemolysis

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.

Hypotonic solution Induced hemolysis: The experiment was carried out with hypotonic solution. The test sample consisted of stock erythrocyte (RBC) suspension (0.5 mL) with 5 mL of hypotonic solution(50mm NaCl) in 10 mm sodium phosphate buffer saline (pH 7.4) containing either the different methanolic extract (2 mg mL^{-1}) or acetyl salicylic acid (0.1 mg mL^{-1}). 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 540 nm using shimadzu UV spectrophotometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

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

Where:

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

OD_2 = Optical density of the test sample in hypotonic solution

Result and discussion of the test sample of Ficusracemosa: The different methanolic extracts of the fruits of concentration 2.0 mg mL^{-1} significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution as compared to the standard acetyl salicylic acid. The roots of *Ficus racemosa* 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 hemolysis of normal condition (Fig. 19 and 12).

Heat induced hemolysis: Aliquots of the isotonic buffer containing 2 mg mL^{-1} of different extractive of *Ficus racemosa* were put into two duplicates centrifuging tube[60]. The vehicle in the same amounts was added to another tube as control. Erythrocyte suspension (30 µL)

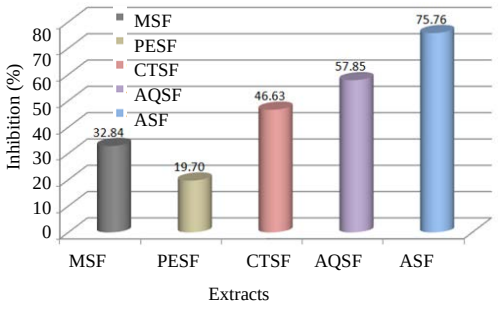

Fig. 19: Hypotonic solution induced hemolysis

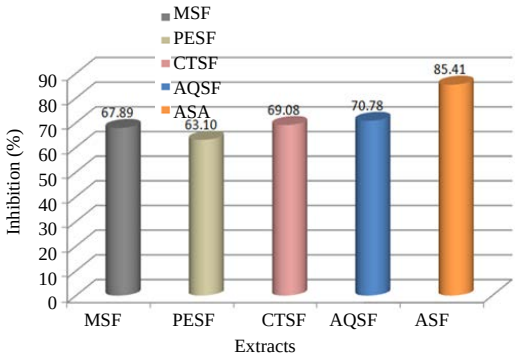

Fig. 20: Heat induced haemolysis

Table 12: Effect of different extractions of Fruits of *Ficus racemosa* of hypnotic solution induced hemolysis of erythrocyte membrane

Sample code	Concentration	Absorbancy	Inhibition (%)
Hypotonic	50 mm	1.675	0
MSF	2 mg mL^{-1}	1.125	32.84
PESF	2 mg mL^{-1}	1.345	19.70
CTSF	2 mg mL^{-1}	0.894	46.63
AQSF	2 mg mL^{-1}	0.706	57.85
ASF	2 mg mL^{-1}	0.406	75.76

was added to each tube and mixed gently by inversion. One pair of tubes was incubated at 54°C for 20 min in a water bath. The other pair was maintained of 0-5°C 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:

$$\text{Inhibition of hemolysis} = 100\times$$
$$\{1\text{-}\times\} = 100\times\{\} = 100\times$$

Where:
OD1 = Test sample unheated
OD2 = Test sample heated and
OD3 = Control sample

The different methanolic extracts of the roots of concentration 2.0 mg mL^{-1} significantly protected the lysis of human erythrocyte membrane induced by heat as compared to the standard acetyl salicylic acid. The fruits of *Ficus racemosa* 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 hemolysis of normal condition (Fig. 20 and Table 13).

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 *Ficus racemosa* plays a significant role in its anti-inflammatory activity may be due to its high flavonoids and tannin content.

EVALUATION OF THROMBOLYTIC ACTIVITY

Introduction: Herbal preparations have been used for the treatment of several diseases since ancient times. The leaves and/or twigs, the stem, the bark and the underground parts of the plants are most commonly used for traditional medicines. Herbal products are often perceived to be safe because they are "natural"[61]. Cerebral Venous Sinus Thrombosis (CVST) is a common disorder associated with high morbidity and mortality[61]. Heparin, an anti-coagulant agent is the first line of treatment for CVST due to its efficacy, safety and feasibility[62]. Thrombolytic drugs such as tissue Plasminogen Activator (t-PA), urokinase, streptokinase, etc. play a key role in the management of CVST patients[63]. The aim of this study was therefore to investigate the thrombolytic activity of methanol extracts and their different fractions of the roots of *Ficus racemosa*.

Table 13: Effect of different extraction of fruits of *Ficus racemosa* of heat induced hemolysis of erythrocyte membrane

Sample code hypotonic	Concentration 50 mm	Unheated (OD1) ----------3.971(OD3)----------	Heated (OD2)	OD3-OD2	OD3-OD1	Inhibition (%)
MSF	2 mg mL^{-1}	0.433	1.569	2.402	3.538	67.89
PESF	2 mg mL^{-1}	0.532	1.801	2.17	3.439	63.10
CTSF	2 mg mL^{-1}	0.278	1.42	2.551	3.693	69.08
AQSF	2 mg mL^{-1}	0.648	1.619	2.352	3.323	70.78
ASA	0.10 mg mL^{-1}	1.16	1.57	2.401	2.811	85.41

Table 14: Thrombolytic Activity (in terms of % of clot lysis) of the extractives of *Ficus racemosa*.

Fractions	Weight of empty vial W_1 (g)	Weight of vial with clot W_2 (g)	Weight of clot $W_3= W_2-W_1$ (g)	Weight of vial after clot lysis W_4 (g)	Weight of lysis clot $W_5 = W_2-W_4$ (g)	Percentage of clot lysis = $100\times W_5/W_3$
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

Materials and methods

Preparation of sample: The thrombolytic activity of all extractives was evaluated using Streptokinase (SK) as a standard substance. About 10 mg of methanol extract and its different fractions of the whole plant of *Ficus racemosa* or taken in different vials to which 1 mL of distilled water was added.

Streptokinase (SK): Commercially produced Altepase (Streptokinase) vial (Beacon Pharma Ltd.) of 15, 00,000 IU was collected and 5 mL of sterile distilled water was added and properly mixed. This suspension was used as a stock from which 100 µL (30,000 IU) was used for thrombolytic *in vitro*.

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.

Thrombolytic activity: Aliquots (5 mL) of venous blood were drawn from healthy volunteers who were distributed in 10 different pre-weighted sterile vials (1 mL/tube) and incubated at 37°C for 45 min. After the formation of the clot, the serum was completely removed without disturbing the clot and each vial containing the clot was again weighed to determine the weight of the clot (clot weight = weight of the clot containing the tube-weight of the tube alone). About 100 µL of aqueous solutions of *Ficus racemosa*, along with crude extracts, were added separately to each vial containing pre-weighed clot. As a positive control, 100 µL of Streptokinase (SK) and as a negative non thrombolytic control, 100 µL of distilled water were added separately to the control vials. All the vials were then incubated at a temperature of 37°C for 90 min 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 % of clot lysis = (wt of released clot/clot weight)×100.

Results and discussion of thrombolytic activity of *Ficus racemosa*: 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 Table 14. Adding 100 M1SK, positive control (3,000,000) to the clot and subsequent incubation at 37°C for 90 min showed that 65.69% of clot lyse on the other hand, distilled water was treated as negative control which showed (10.23%) a negligible percentage between positive and negative control was found to be very significant. In this study, thrombolytic activity among all *Ficus racemosa* rot extractives was found to be negligible. Thrombolytic activity levels were present in the Methanol soluble fraction (MESF 32.91%), the Pet-Ether soluble fraction (PESF 34.27%), the carbon tetra chloride soluble fraction (CTCSF 30.70%) and the dichloromethane soluble fraction (CSF 38.83%) and the Aqueous soluble fraction (AQSF 31.18%) (Fig. 21 and Table 14).

From this experiment, it can be concluded that the extractives of *Ficus racemosa* showed less clot lysis activity than the standard Substances treptokinase (SK).

EVALUATION OF PERIPHERAL ANALGESIC ACTIVITY

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

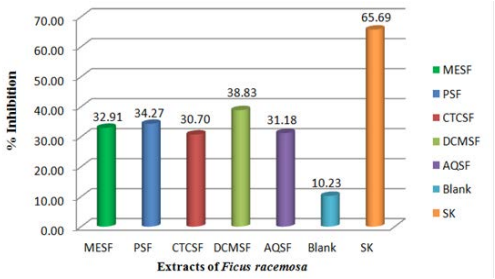

Fig. 21: Thrombolytic Activity (in terms of % of clot lysis) of extractives of *Ficus racemosa*; Thrombolysis of different extracts of *ficus recemosa*

(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.

Principle: Peripheral analgesic activity can be evaluated by acetic acid induced writhing method[64]. 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[65]. According to this principle the crude methanolic extract of roots of *Fecus racemosa* was subjected to analgesic testing at two different doses: Dose 200 and 400 mg kg^{-1} of body weight.

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 ± 2°C; 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

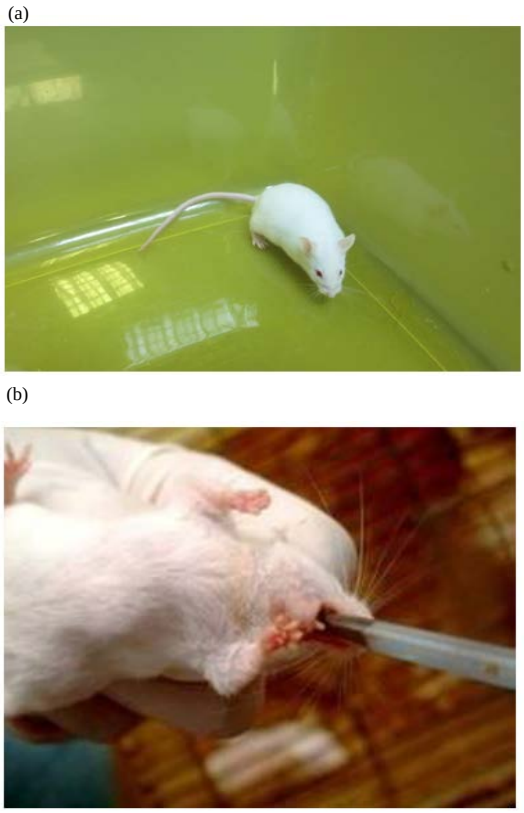

Fig. 22(a, b): Feeding experimental animal (a) Swiss albino mice (a) and (b) Oral administration

environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Experimental design: Twelve experimental animals were randomly selected and divided into four groups denoted as group-I-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 (Fig. 22) and marked as M-1 = Mice 1, M-2 = Mice 2, M-3 = Mice 3.

Preparation of test materials: In order to administer the extract at doses of 400 mg kg^{-1} body wt and 200 mg kg^{-1} 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

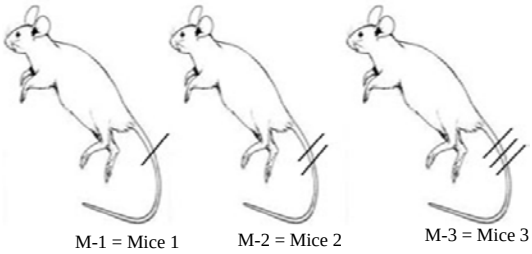

Fig. 23: Numbering of mice

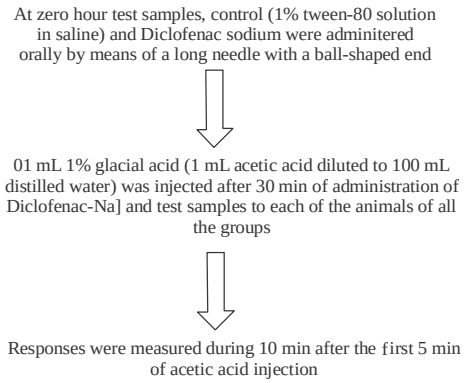

Fig. 24: Schematic representation of procedure for screening of analgesic property

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^{-1}-body weight was made (Fig. 23 and 24).

Procedure

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 min commencing just after the subcutaneous administration of formalin solution (Fig. 25 and Table 15, 16).

Data collection: 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 *Fecus racemosa* at dose of 400 mg kg^{-1} exhibited significant peripheral analgesic activity and at doses of 200 mg kg^{-1} also showed peripheral analgesic activity to a lesser extent compared to Diclofenac Na (Table 17).

Statistical evaluation of the data obtained after the administration of the samples confirmed that the

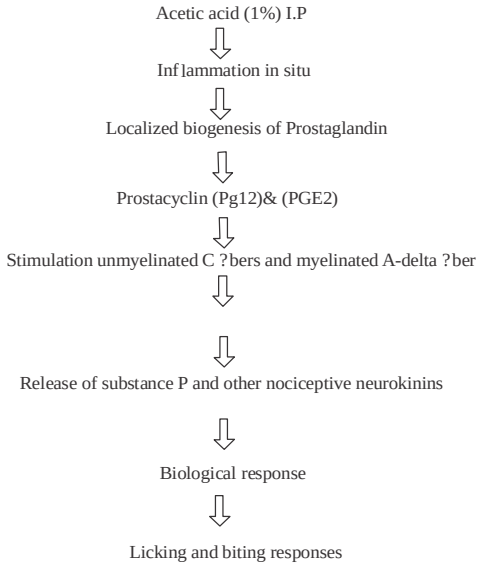

Fig. 25: Mechanism of action of pain induction

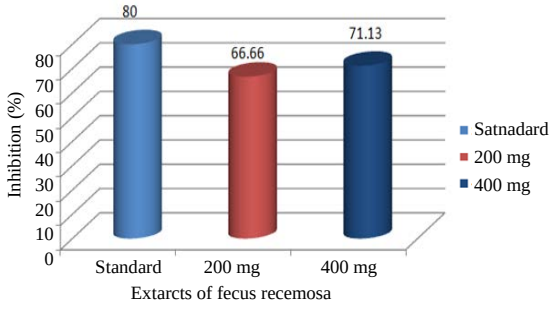

Fig. 26: Comparison of %inhibition of licking responses by Methanolic extract of *Fecus racemosa;* Analgesic activity of flowers extracts of Fecus racemosa

Methanolic extract of roots of *Fecus racemosa* at doses of 200 and 400 mg kg^{-1} were showed a percent Inhibition of 4.55 and 9.09%, respectively. Both data was extremely statistically significant (Fig. 26).

Results 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 and 400 mg kg^{-1} 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^{-1} dose exhibited maximum inhibition of licking and biting 71.13%.

Table 15: Test materials used in the evaluation of analgesic activity of flowers of fecus racemosa

Code no.	Test Samples	Group	Identification	Dose (mg kg^{-1})*
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 16: Screening of analgesic activity by counting the time of licking and biting after subcutaneously administration of 5% acetic acid

	Writhing count							
Animal group	M-1	M-2	M-3	Average	SD	SE	Writhing (%)	Inhibition (%)
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 fecus racemosa at dose of 400 mg/kg exhibited significant peripheral analgesic activity and at doses of 200 mg/kg also showed peripheral analgesic activity to a lesser extent compared to Diclofenac Na

Table 17: Statistical evaluation for the peripheral analgesic activity test

Code no.	Writhing (%)	Inhibition (%)	t-values	p-values	Level of significance
Standard (diclofenac)	-	80	11.0322	0.004058	Very statistically significant
MESF (200 mg)	-	66.66	18.3870	0.001472	Very statistically significant
(MESF 400 mg)	-	71.13	10.4164	0.004546	Very statistically significant

EVALUATION OF CENTRAL ANALGESIC ACTIVITY

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°C .The reaction time is the time required by the mice to deflect their tails. The time required to withdraw the tail was recorded.

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±2°C; 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 h before and during the experiment. The ethics for use of experimental animals were followed carefully.

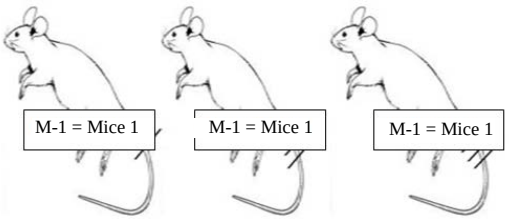

Fig. 27: Numbering of mice

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 (Fig. 27) and marked as M1 = Mice 1, M2 = Mice 2 and M3 = Mice 3.

Table 18: Test materials used in the evaluation of central analgesic activity of crude extract and its different fractions of ficusb racemosa.

Code No.	Test samples	Groups	Identification	Dose (mg kg^{-1})*	Route of administration
Control	Distilled Water	I	Control group	50 mL	Oral
Standard	Diclofenac Na	II	Standard group	2 mg	Oral
MESF 200 mg	Methanolic extract	III A	Test sample	200 mg	Oral
MESF 400 mg	Methanolic extract	III B	Test sample	400 mg	Oral

Table 19: Analysis of the data obtained after 30 min

	Immersion time count (sec)						
Animal groups	M-1	M-2	M-3	Average time of immersion	SD	SE	Elongation (%)
Control (Water)	2.62	2.56	2.78	2.65	0.14	0.099	-
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

Table 20: Statistical evaluation of the data obtained after 30 min

Groups	t-test values	Degree of freedom	p-values	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

Materials and equipment:

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

Experimental procedure

Preparation of test materials: In order to administer the extract at doses of 400 mg kg^{-1} body wt and 200 mg kg^{-1} 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 2 mg kg^{-1} for 32 mg mice.

Methodology: Test samples and control were given orally by means of a feeding needle to the mice at 0 h. At 0 h, 1-2 cm of the tail of mice was immersed in warm water kept constant at 550°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 sec 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 min after the administration of drugs. A 30 min interval was given to ensure proper absorption of the administered substances. Then diclofenac-Na solution was administered subcutaneously to the mice. After 30, 60 and 90 min, 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 (Table 18-20):

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

According to the data in the above table the methanol soluble fraction at dose of 400 mg kg^{-1} exhibited highly significant central analgesic activity.

The crude extracts at doses of 400 mg kg^{-1} also exhibited significant central analgesic activity after 30 min compared to diclofenac Na. The MESF at doses of 200 mg kg^{-1} also showed central analgesic activity to a lesser extent.

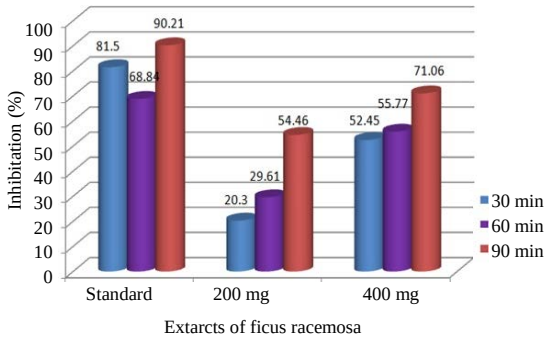

Fig. 28: Comparison of percent time elongation of tail immersion of different samples; Central analgesic activity by tail immersion method of *Ficus recemosa*

Statistical evaluation of the data obtained 30 min after the administration of the samples confirmed that the methanolic extract at doses of 400 mg kg^{-1} exhibited significant central analgesic activity. The crude at doses of 200 mg kg^{-1} showed central analgesic activity to a lesser extent.

In the table M-1 = mice 1, M-2 = mice 2, M-3 = mice 3. The data in Table 18 showed that after 60 min the central analgesic action of Crude Methanol extract at a dose of 400 mg kg^{-1} became stronger. They showed a percent elongation of 55.77 and 29.61% at a dose of 400 mg and 200 mg kg^{-1} bode weight. But the central analgesic activity of aqueous soluble fraction at 400 mg kg^{-1} dose still remains highly significant after 60 min (Table 20-28).

In the table M-1 = mice 1, M-2 = mice 2, M-3 = mice 3. The data in Table 19 showed that after 90 min the central analgesic action of Crude Methanol extract at a dose of 400 mg kg^{-1} became stronger. It showed a percent elongation of 71.06%. At a dose of 200 mg kg^{-1} showed a percent elongation of 54.46%. The statistical evaluation of the data showed that the central analgesic activity of the samples declined after 90 min (Fig. 28):

- 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^{-1} dose
- The central analgesic activity is highest after 90 min
- As the time progress the analgesic activity decreases After 90 min there is almost no central analgesic activity in the plant extracts

EVALUATION OF HYPOGLYCEMIC ACTIVITY

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 *Ficus racemosa* at 250 and 500 mg kg^{-1} doses were examined ompared with relative to that of control and standard group. Here, Glibenclamide was used as a standard drug.

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.

Experimental design: Twelve experimental animals were randomly selected and divided into ten groups denoted as group-I-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.

Table 21: Analysis of the data obtained after 60 min

Animal groups	Immersion time count (seconds) M-1	M-2	M-3	Average time of immersion	SD	SDE	Elongation (%)
Control (Water)	2.97	2.19	2.64	2.60	0.184	0.130	-
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

Table 22: Statistical evaluation of the data obtained after 60 min

Groups	t-test value	Degree of freedom	p-values	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 23: Analysis of the data obtained after 90 min

Animal groups	Immersion time count (seconds) M-1	M-2	M-3	Average time of immersion	SD	SDE	Elongation (%)
Control (Water)	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

Table 24: Statistical evaluation of the data obtained after 90 min

Group	t-test value	Degree of freedom	p-values	Level of significance
Standard (Diclofenac)	29.2161	2	0.000585	Very statistically significant
MESF (200 mg)	10.7110	2	0.004302	Very statistically significant
MESF (400 mg)	14.8779	2	0.002244	Very statistically significant

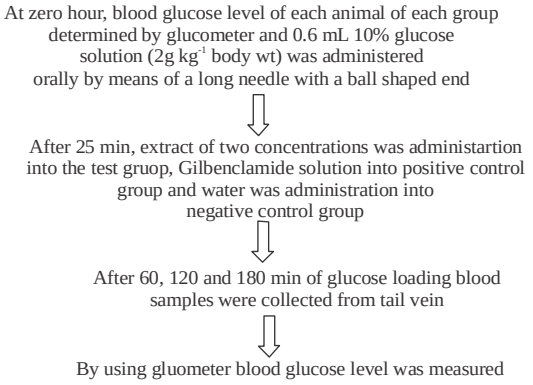

Fig. 29: Schematic representation for determining blood glucose root of mice after administration of *Ficus racemosa* extract

Fig. 30: Pricking of mice's tail

The animals were individualized in the following way (Fig. 29) and marked as M-1 = Mice 1, M-2 = Mice 2 and M-3 = Mice 3.

Preparation of test materials: In order to administer the extract at doses of 400 mg kg^{-1} body wt and 200 mg kg^{-1} 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^{-1} body weight, 10 mg tablet was dissolved into 3.0 mL distilled water (Fig. 29-31).

Procedure

Result and discussion of hypoglycemic activity of test materials of *Ficus racemosa*: The effects of methanolic extract of stem roots of *Ficus racemosa* at 200 and 400 mg kg^{-1} dose and its different fractionates at 200 mg kg^{-1} doses to lower blood glucose level were observed as follows to evaluate their hypoglycemic activity.

The methanolic extrat of roots of *Ficus racemos* a has statistically significant blood glucose lowing activity

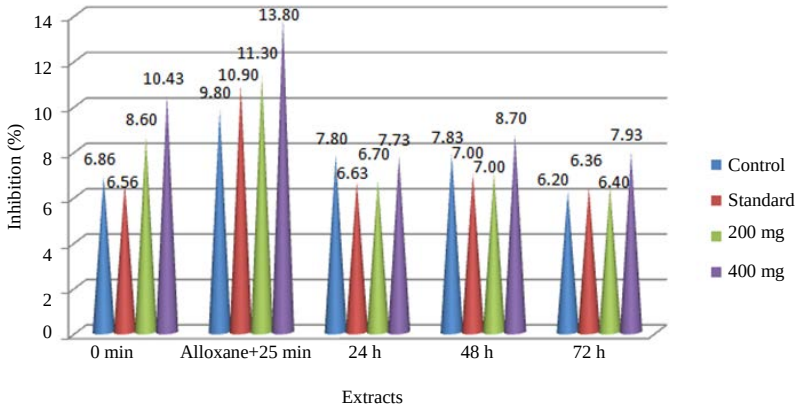

Fig. 31: Plasma level of glucose of different groups of mice at different times; Hypoglycemic activity of methanoilc extracts of *Ficus racemosa*

Table 25: Test materials used in the evaluation of hypoglycemic activity of crude extract and its different fractions of *Ficusracemosa*

Code no.	Test samples	Groups	Identification	Dose (mg kg^{-1})*
CTL	1% Tween-80 and DMSO in			
normal saline	I	Control group	0.1 mL 10 g of body wt	
STD	Glibenclamide	II	Standard group	10
MESF 200 mg	Methanolic extract of *Ficus racemosa*	III A	Test sample	200
MESF 400 mg	Methanolic extract of *Ficus racemosa*	III B	Test sample	400

Table 26: Plasma level of glucose (mmol L^{-1}) of mice at different times

	0 Min		Glucose+25 min		24 h		48 h		72 h	
Groups	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	7.80	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	6.70	5.5	7	6.3	6.4
M-2	8.6	-	11.6	-	7.0	-	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	-	7.0	-	6.9	-	7.7	-
M-3	10	-	11.9	-	7.4	-	8.7	-	8.3	-

Table 27: Hypoglycemic activity of crude extract and its different fractions of *Ficusracemosa*

	Plasma level of glucose (Mean)							
Code	0 min	Glucose+ 25 min	24 h	48 h	72 h	Mean	SD	SE
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 28: Statistical evaluation of the data

Code	SE	t-test value	Degree of freedom	p-values	Level of significance
Control	-	-	4	-	-
Standard	-	21.8103	4	0.000013	Extremely statistically significant
200 mg	-	22.0302	4	0.000013	Extremely statistically significant
400 mg	-	21.5827	4	0.000014	Extremely statistically significant

at dose of 400 mg kg^{-1}. 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 (Fig. 31).

The methanolic extrat of roots of *Ficus racemos* a has statistically significant blood glucose lowing activity at dose of 400 mg kg^{-1}. 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 (Fig. 31).

CONCLUSION

All the conducted experiments in the present study which are based on not only cured extract but also many different kinds of soluble frictions are considered to be initially and more sophisticate research is important to gain a concrete conclusion about the findings of the present study. It can be concluded from the above findings, the plant Ficus recaemosa have moderate to significant thrombolytic, membrane stabilizing, antioxidant, antimicrobial, analgesic and hypoglycemic activity. So, further scientific studies are necessary to explain detailed not only mechanism of action but also isolation the responsible active principles.

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