CYTOTOXIC & ANTI-OXIDANT ACTIVITY OF ETHYL ACETATE (EA) FRACTION OF *Ficus hispida* LEAF EXTRACT

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

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DECLARATION BY THE CANDIDATE

I, Jannatul Ferdous Mitu, hereby declare that this dissertation, entitled " Cytotoxic & Anti-oxidant Activity of Ethyl Acetate (EA) Fraction of *F. hispida* Leaf Extract" submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me under the guidance of Abdullah-Al-Faysal, Senior Lecturer, Department of Pharmacy, East West University, Dhaka. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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This is to certify that the dissertion, entitled **"Cytotoxic & Anti-oxidant Activity of Ethyl Acetate (EA) Fraction of *F. hispida* Leaf Extract"** is a bonafide research work done, under our guidance and supervision by Jannatul Ferdous Mitu (ID: 2013-3-70-014), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Dedication

This Research paper is dedicated to

My beloved Parents,

Who are my Biggest Inspiration…

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ABSTRACT

The study was designed for pharmacological investigation of ethyl acetate (EA) fraction of methanol extract of the leaves of *Ficus hispida* (Family: Moraceae). The powdered leaves of *Ficus hispida* were extracted with methanol and then partitioned with n-hexane, dichloromethane and ethyl acetate consecutively. The EA fraction remaining was investigated for total flavonoid content, total phenol content, brine shrimp lethality test. The fraction contained 139.167mg AAE/gm of dried extract in total phenolic content assay and 9.73mg quercetin/gm of dried extract in total flavaniod content assay. Screening for cytotoxic properties using brine shrimp lethality bioassay with tamoxifen (LC_{50} value of 35.23 µg/ml) as positive control showed that the fraction have considerable cytotoxic potency exhibiting LC_{50} value of 1.79 µg/ml. The EA fraction showed strong cytotoxic activity, low antioxidant activity and low to moderate antimicrobial activity. Further investigations are needed for the proper identification and isolation of these bioactive compounds to produce safer drugs for treatment of harmful diseases.

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

CHAPTER ONE

INTRODUCTION

Introduction

1.1 Overview

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis. Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again.

1.2 Historical Sources Relevant For Study of Medicinal Plants' Use

1.2.1 Ancient Times:

- The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.
- The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: Rhei rhisoma, camphor, Theae folium, Podophyllum, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra.
- The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc.
- In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to.

Some of them were given the names after mythological characters from these epics; for instance, Elecampane (Inula helenium L. Asteraceae) was named in honor of Elena, who was the centre of the Trojan War. As regards the plants from the genus Artemisia, which were believed to restore strength and protect health, their name was derived from the Greek word artemis, meaning "healthy."

- Herodotus (500 BC) referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (Scilla maritima), mustard, and cabbage.
- The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (Centaurium umbellatum Gilib) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as adstringents.
- Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth.
- In the description of the plant toxic action, Theophrast underscored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants. In his work "De re medica" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc.
- In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica."
- This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic materia medica until the late Middle Ages and the

Renaissance.Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect. In addition to the plant description, the names in other languages coupled with the localities where they occur or are grown are provided. The plants having mild effect are dominant, but there are also references to those containing alkaloid or other matter with strong effect (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade).

- Dioscorides' most appreciated domestic plants are as follows: willow, camomile, garlic, onion, marsh mallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore). Camomile (Matricaria recucita L.), known under the name Chamaemelon, is used as an antiphlogistic to cure wounds, stings, burns, and ulcers, then for cleansing and rinsing the eyes, ears, nose, and mouth. Owing to its mild carminative action, it is particularly appropriate for usage with children. Dioscorides deemed that it had abortive action, on which he wrote, "The flower, root, and the entire plant accelerate menstruation, the release of the embryo, and the discharge of urine and stone, provided that they are used in the form of an infusion and baths." This untrue belief was later embraced by both the Romans and the Arabs; hence the Latin name Matricaria, derived from two words: mater denoting "mother," i.e. matrix, denoting 'uterus'. Dioscorides differentiated between a number of species from the genus Mentha, which were grown and used to relieve headache and stomach ache. The bulbs of sea onion and parsley were utilized as diuretics, oak bark was used for gynaecological purposes, while white willow was used as an antipyretic.
- As maintained by Dioscorides, Scillae bulbus was also applied as an expectorant, cardiac stimulant, and antihydrotic.It is worth underscoring that Dioscorides pointed to the possibility of forgery of drugs, both the domestic ones such as opium forged by a yellow poppy (Glaucium flavum) milk sap and poppy, and the more expensive oriental drugs, transported by the Arab merchants from the Far East, such as iris, calamus, caradmomum, incense, etc.
- Pliny the Elder (23 AD-79), a contemporary of Dioscorides, who travelled throughout Germany and Spain, wrote about approximately 1000 medicinal plants in his book

"Historia naturalis." Pliny's and Dioscorides' works incorporated all knowledge of medicinal plants at the time.

- The most distinguished Roman physician (concurrently a pharmacist), Galen (131 AD–200), compiled the first list of drugs with similar or identical action (parallel drugs), which are interchangeable—"De succedanus." From today's point of view, some of the proposed substitutes do not correspond in a pharmacological context and are absolutely unacceptable. Galen also introduced several new plant drugs in therapy that Dioscorides had not described, for instance, Uvae ursi folium, used as an uroantiseptic and a mild diuretic even in this day and age.

1.2.2 Middle Ages:

- In the Middle Ages, the skills of healing, cultivation of medicinal plants, and preparation of drugs moved to monasteries. Therapy was based on 16 medicinal plants, which the physicians-monks commonly grew within the monasteries as follows: sage, anise, mint, Greek seed, savory, tansy, etc.
- In the seventh century AD the Slavic people used Rosmarinus officinalis, Ocimum basilicum, Iris germanica, and Mentha viridis in cosmetics, Alium sativum as a remedy and Veratrum album, Cucumis sativus, Urtica dioica, Achilea millefolium, Artemisia maritime L., Lavandula officinalis, Sambuci flos against several injurios insects, i.e. louses, fleas, moths, mosquitos, and spiders and Aconitum napellus as a poison in hunting.
- Charles the Great (742 AD–814), the founder of the reputed medical school in Salerno, in his "Capitularies" ordered which medicinal plants were to be grown on the state-owned lands. Around 100 different plants were quoted, which have been used till present days such as sage, sea onion, iris, mint, common centaury, poppy, marsh mallow, etc. The great emperor especially appreciated the sage (Salvia officinalis L.). The Latin name of sage originates from the old Latins, who called it a salvation plant (salvare meaning "save, cure"). Even today sage is a mandatory plant in all Catholic monasteries.The Arabs introduced numerous new plants in pharmacotherapy, mostly from India, a country they used to have trade relations with, whereas the majority of the plants were with real medicinal value, and they have persisted in all pharmacopoeias in the world till today.

The Arabs used aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna, and so forth. Certain drugs with strong action were replaced by drugs with mild action, for instance, Sennae folium was used as a mild laxative, compared to the purgatives Heleborus odorus and Euphorbium used until then.

- Throughout the Middle Ages European physicians consulted the Arab works "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980-1037), and "Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248), in which over 1000 medicinal plants were described. For Macedonia, St Clement and St Naum of Ohrid's work are of particular significance. They referred to the Nikeian pharmacological codex dating from year 850, and transferred his extensive knowledge on medicinal plants to his disciples and via them to the masses.
- Marco Polo's journeys (1254-1324) in tropical Asia, China, and Persia, the discovery of America (1492), and Vasco De Gama's journeys to India (1498), resulted in many medicinal plants being brought into Europe.

1.2.3 Early Modern:

- Botanical gardens emerged all over Europe, and attempts were made for cultivation of domestic medicinal plants and of the ones imported from the old and the new world. With the discovery of America, materia medica was enriched with a large number of new medicinal plants: Cinchona, Ipecacuanha, Cacao, Ratanhia, Lobelia, Jalapa, Podophylum, Senega, Vanilla, Mate, tobacco, red pepper, etc.
- In 17th century, Cortex Chinae, yielded from quinine bark Cinchona succirubra Pavon, under the name countess' powder, since the Countess of Chinchon was the first one who used it, was introduced to European medicine. Quinine bark rapidly overwhelmed England, France, and Germany despite the fact that there was many an opponent to its use among distinguished physicians—members of a range of academies.
- Paracelsus (1493-1541) was one of the proponents of chemically prepared drugs out of raw plants and mineral substances; nonetheless, he was a firm believer that the collection of those substances ought to be astrologically determined. He continuously emphasized his belief in observation, and simultaneously supported the "Signatura doctrinae"—the signature doctrine. According to this belief, God designated his own sign on the healing

substances, which indicated their application for certain diseases. For example, the haselwort is reminiscent of the liver; thus, it must be beneficial for liver diseases; St John's wort Hypericum perforatum L. would be beneficial for treatment of wounds and stings given that the plant leaves appear as if they had been stung.

- While the old peoples used medicinal plants primarily as simple pharmaceutical forms—infusions, decoctions and macerations—in the Middle Ages, and in particular between 16th and 18th centuries, the demand for compound drugs was increasing. The compound drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug the theriac was produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively.
- In 18th century, in his work Species Plantarium (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. The species were described and named without taking into consideration whether some of them had previously been described somewhere. For the naming, a polynomial system was employed where the first word denoted the genus while the remaining polynomial phrase explained other features of the plant (e.g. the willow Clusius was named Salix pumila angustifolia antera). Linnaeus altered the naming system into a binominal one. The name of each species consisted of the genus name, with an initial capital letter, and the species name, with an initial small letter.

1.2.4 19th and 20th Centuries:

- Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy.
- With the upgrading of the chemical methods, other active substances from medicinal plants were also discovered such as tannins, saponosides, etheric oils, vitamins, hormones, etc.In late 19th and early 20th centuries, there was a great danger of elimination of medicinal plants from therapy. Many authors wrote that drugs obtained from them had many shortcomings due to the destructive action of enzymes, which cause

fundamental changes during the process of medicinal plants drying, i.e. medicinal plants' healing action depends on the mode of drying.

- In 19th century, therapeutics, alkaloids, and glycosides isolated in pure form were increasingly supplanting the drugs from which they had been isolated. Nevertheless, it was soon ascertained that although the action of pure alkaloids was faster, the action of alkaloid drugs was full and long-lasting. In early 20th century, stabilization methods for fresh medicinal plants were proposed, especially the ones with labile medicinal components. Besides, much effort was invested in study of the conditions of manufacturing and cultivation of medicinal plants.
- On account of chemical, physiological, and clinical studies, numerous forgotten plants and drugs obtained thereof were restored to pharmacy: Aconitum, Punica granatum, Hyosciamus, Stramonium, Secale cornutum, Filix mas, Opium, Styrax, Colchicum, Ricinus, and so forth. The active components of medicinal plants are a product of the natural, most seamless laboratory. The human organism accepts the drug obtained from them best in view of the fact that man is an integral part of nature.There are scores of examples of this kind; perhaps they will instigate serious research into the old manuscripts on medicinal plants, which would not be observed out of curiosity about history but as potential sources of contemporary pharmacotherapy.

1.2.5 21th Century:

- In present days, almost all pharmacopoeias in the world—Ph Eur 6, USP XXXI,BP 2007—proscribe plant drugs of real medicinal value. There are countries (the United Kingdom,Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine).
- Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Plant drugs and

phytopreparations, most commonly with defined active components, verified action and, sometimes, therapeutic efficiency, are applied as therapeutic means.

- In the major European producer and consumer of herbal preparations—Germany, rational phytotherapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs.
- With the new Law on Drugs and Medical Devices dated September 2007and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs. Herbal substances may also be utilized for manufacture of homeopathic drugs, which are stipulated in the current law, too. In the Republic of Macedonia herbal preparations are dispensed without a medical prescription, as "over the counter" (OTC) preparations. (Petrovska, 2012)

1.3 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- Synergic medicine: The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- Support of official medicine: In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.
- Preventive medicine: It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Singh, 2015)

1.4 Classification of Medicinal Plants

Classification of medicinal plants is organized in different ways depending on the criteria used. In general, medicinal plants are arranged according to their active principles in their storage

organs of plants, particularly roots, leaves, flowers, seeds and other parts of plant. These principles are valuable to mankind in the treatment of diseases. Reports on the classification of many plant species yielding vegetable oils used in cosmetics and body and skin care preparations are sporadic or lacking. Herbs are classified in many ways. Some of them are:

- According to the usage;
- According to the active constituents;
- According to the period of life;
- According to their taxonomy;
- According to their habitats.

1.4.1 Classification according to the usage

The herbs are classified in four parts:

Figure 1.1: Lemon balm

i. Medicinal Herbs: Medicinal herbs have curative powers and are used in making medicines because of their healing properties like marigold, lemon balm, lavender, johnny-jump-up, feverfew etc.

ii. Culinary Herbs: Culinary herbs are probably the mostly used as cooking herbs because of their strong flavours like oregano, parsley, sweet basil, horseradish, thyme etc.

Figure 1.2: Parsley

Figure 1.3: Basil

iii. Aromatic Herbs: Aromatic herbs have some common uses because of their pleasant smelling flowers or foliage. Oils from aromatic herbs can be used to produce perfumes, toilet water, and various scents. For e.g. mint, rosemary, basil etc.

iv. Ornamental Herbs: Ornamental herbs are used for decoration because they have brightly coloured flowers and foliage like lavender, chives, bee balm, lemongrass etc.

Figure1.4: Lemongrass

1.4.2 Classification according to the active constituents

According to the active constituents all herbs are divided into five major categories:

Figure 1.5: Fennel

i. Aromatic Herbs: The name is a reflection of the pleasant odour that many of these herbs have. They are used extensively both therapeutically and as flavourings and perfumes. Aromatic herbs are divided into two subcategories: stimulants and nervines.

ii. Astringent Herbs: Tannins in Astringent Herbs have the ability to precipitate proteins, and this "tightens," contracts, or tones living tissue, and helps to halt discharges. They affect the digestive, urinary, and circulatory systems, and large doses are toxic to the liver. They are analgesic, antiseptic, ant abortive, astringent, emmenagogue, hemostatic, and styptic. For e.g. peppermint, red raspberry.

iii. Bitter Herbs: Bitter Herbs are named because of the presence of phenols and phenol glycosides, alkaloids, or saponins, and are divided into four subcategories: laxative herbs, diuretic herbs, saponin containing herbs, and alkaloid containing herbs.

iv. Mucilaginous Herbs: Mucilaginous herbs derive their properties from the polysaccharides they contain, which give these herbs a slippery, mild taste that is sweet in water. All plants produce mucilage in some form to store water and glucide as a food reserve.

Figure 1.6: Fenugreek

They eliminate the toxins from the intestinal system, help in regulating it and reduce the bowel transit time. They are antibiotic, antacid, demulcent, emollient, vulnerary, and detoxifier in nature. For e.g. althea, aloe, burdock, comfrey, dandelion, Echinacea, fenugreek, kelp, psylium, slippery elm, dulse, glucomannan from Konjak root, Irish moss, and mullein.

Figure 1.7: Carrot

v. Nutritive Herbs: These herbs derive both their name and their classification from the nutritive value they provide to the diet. They are true foods and provide some medicinal effects as fibber, mucilage, and diuretic action. But most importantly they provide the nutrition of protein, carbohydrates, and fats, plus the vitamins and minerals that are necessary for adequate nutrition. For e.g. rosehips, acerola, apple, asparagus, banana, barley grass, bee pollen, bilberry, broccoli, cabbage, carrot, cauliflower, grapefruit, hibiscus, lemon, oat straw, onion, orange, papaya, pineapple, red clover, spirulina, stevia, and wheat germ.

1.4.3 Classification according to the period of life

Herbs also can be classified as annuals, biennials, and perennials. Annuals bloom one season and then die. Biennials live for two seasons, blooming the second season only. Once established, perennials live over winter and bloom each season. They can last for many years with proper care.

Figure 1.8: Saffron

i. Annual Herbs: They complete their life cycle in one year; start them from seed. The annuals have to be seeded each year unless conditions are favorable enough in the garden to seed themselves. Annual herbs include: Anise, Basil, Borage, Calendula (Pot Marigold), Chamomile, Chervil, Cilantro/ Coriander, Dill Bouquet, Dill Dukat, Fennel smoky, Marjoram, Parsley, Saffron, Summer Savoury.

ii. Perennial Herbs: They grow for more than one season and include sweet marjoram, parsley, mint, sage, thyme and chives. Most can be started from young plants. Perennial herbs include: Alfalfa, Allspice, Aloe Vera, Angelica, Bee Balm, Bay leaves, Catnip, Chives Common, Lavender, Lemon Balm, Mints (Spearmint, peppermint, apple mint, orange mint), Mitsuba, Oregano, Rosemary, Sorrel, Salad Burnet, Sage, Tarragon, Thyme, Watercress, Yarrow.

iii. Biennial Herbs: They are plants which live two season and bloom in the second season only. They are Caraway seeds, Prime rose, Bai Zhi, Mullein, Teasel, Viper's Bugloss. Like all other plants, herbs can be propagated from seeds, cuttings, divisions, and to a lesser degree, layering.

Figure 1.9: Prime rose

1.4.4 Classification according to their taxonomy

Botanists have developed this system into a comprehensive diversely branched family tree of classifications, which includes all known plants. The complete ascending sequence is species, genus, family, order, class and division. The meaning of the botanical name may be indicative of the history of the plant i.e. a genus may be named after a particular botanist.

For e.g.; the Kaempferia, is named after the German physician Englebert Kaempfer 1651-1716. The name may also tell something of the habit or morphological characteristics of the plant e.g. in *Gaultheria procumbens* L., the latter name derives from _procumbent‘ which describes the plant‘s habit.

1.4.5 Classification according to their habitats

The Earth has many different environments, varying in temperature, moisture, light, and many other factors. Each of these habitats has distinct life forms living in it, forming complex communities of interdependent organisms. A complex community of plants and animals in a region and a climate is called a biome.

1.5 The Healing Power of Herbs

Figure 1.10: Herbs

Traditional cures from plants and herbs have been used by herbalists and apothecaries throughout the centuries. Herbs do more than simply adding flavour and colour to favourite dishes, their healing and restorative powers are pretty impressive too...

According to the UK's leading organic herb grower Jekka McVicar the healing power of herbs is grossly underestimated: "We are what we eat. We don't doubt that fruits and vegetables, seeds and nuts contain a range of vitamins, minerals and cancer-fighting properties, yet the nutrient content and medicinal properties of herbs are often overlooked."

With that in mind, here's the lowdown on herbs and how they may help:

- **To ease digestion:**

Often it is only when herbs are heated that their full aroma is released - that's what makes mouth water. This aids the release of saliva, which prepares stomach for food. It's the enzymes in saliva that trigger the digestive process, helping the body to break down fats and starches. If this doesn't happen before food reaches the stomach, then it isn't processed properly and digestive problems such as bloating, constipation, diarrhoea, wind and irritable bowel may result.

What to use: Thyme, rosemary, oregano, mint.

- **Herbs with anti-cancer properties:**

Figure 1.11: Green tea

Many herbs contain flavonoids; nutrients widely available in fruits and vegetables and thought to help prevent cancer and reduce the risk of heart attacks and strokes. According to Dr Winston Craig, Professor of Nutrition at Andrews University in the United States, flavonoids help vitamin C work more efficiently as an antioxidant, mopping up the free radicals that cause cancer.

What to use: Onions, rosemary, sage, thyme, chamomile, dandelion, ginkgo, green tea, milk thistle.

- **To help prevent tumours:**

Some herbs contain phytochemicals called terpenoids which are potent antioxidants, thought to inhibit the growth of tumours.

What to use: Caraway, spearmint, dill, coriander, lavender, rosemary, sage, thyme, lemongrass, chamomile, basil, rosemary, mint, cardamom, celery seed, fennel and peppermint.

- **As natural antiseptics:**

There is anecdotal evidence to suggest that some herbs have antiseptic qualities.Jekka McVicar says: "Before refrigerators were invented, large households stored cold meats in their cellars, covered in salt and wrapped in fresh sage leaves to preserve it. After shooting, fresh game was left to hang to tenderise along with bunches of fresh thyme, not only to add flavour, but also because thyme's antiseptic properties helped prevent stomach upsets when the game was eaten."

Figure 1.12: Thyme

What to use: Thyme, sage, rosemary and bay leaves

- **To boost the immune system:**

Herbs high in flavonoids may also have mild anti-inflammatory properties. Garlic is known to be good for the immune system and may stimulate cells which attack invading organisms. Echinacea is the best-known herb thought to have immune boosting qualities. It stimulates the immune system promoting the activity of lymphocytes - types of cells which circulate in the body ready to eliminate foreign 'invaders' such as viruses.

Figure 1.13: Onion

What to use: Onions, rosemary, sage, thyme, chamomile, dandelion, ginkgo, green tea and milk thistle

- **To promote heart health:**

Garlic,like onion, is not normally thought of as a herb but according to Jekka McVicar it is one. Research suggests garlic may protect against heart attacks and strokes because it helps lower bad cholesterol.Substances called catechins have also been shown to have cholesterol-reducing properties.

What to use: garlic, green tea

- **Reduce cholesterol:**

Some herbs contain anthocyanins - the pigments responsible for the red, pink, purple, and blue shades of some fruit and flowers. Anthocyanins can also help reduce the formation of harmful cholesterol, so they may provide some protection.

What to use: rosehip tea

Figure 1.14: Rosehip tea

- **Herbs which may heal:**

Many herbs are reputed to have healing qualities. Jekka McVicar keeps a pot of aloe vera on her kitchen windowsill as she's prone to burning herself when cooking. She just breaks off a leaf and rubs the glutinous gel on the burn to help prevent blistering. Jekka suggests making your own teas with one teaspoon of dried or two teaspoons of fresh herbs per cup of freshly boiled water.

What to use: Chamomile for insomnia; dill or peppermint for indigestion; elderflower for relief from a cold; lemon balm for tension and headaches; rosemary to improve concentration and bad breath. (Lewin, 2014).

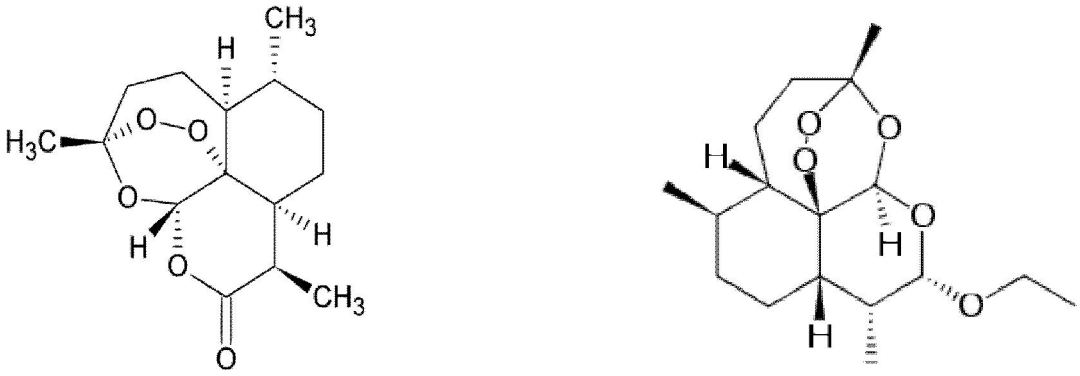
1.6 Drug Discovery from Medicinal Plants

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (i.e traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country where plant(s) of interest are collected. Phytochemists (natural product chemists) prepare extracts from the plant material, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science.

Numerous methods used to acquire compounds for drug discovery include:

- isolation from plants and other natural sources;
- synthetic chemistry;
- combinatorial chemistry, and
- molecular modeling.

Despite the recent interest. in molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, the natural products, and particularly that of medicinal plants, remain an important source of new drugs, drug leads, and chemical entities. In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or were derived from natural products. An example is Arteether (Fig 1.15, a potent antimalaria drug. It is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* (Asteraceae), a plant used in traditional Chinese medicine (TCM).



Artemisinin Arteether

Figure 1.15: The structure of artemisinin and arteether.

Despite evident successes of drug discovery from medicinal plants, future endeavors face many challenges. Pharmacognosists, phytochemists, and other natural product scientists will need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts. The process of drug discovery has been estimated to take an average of 10 years upwards and cost more than 800 million US dollars. Much of this time and money is spent on the numerous leads that are discarded during the drug discovery process. It has been estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use. Lead identification is only the first step in a lengthy drug development process. There is also lead optimization (involving medicinal and combinatorial chemistry), development (including toxicology, pharmacology, pharmacokinetics,

ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials which all take a considerable length of time.

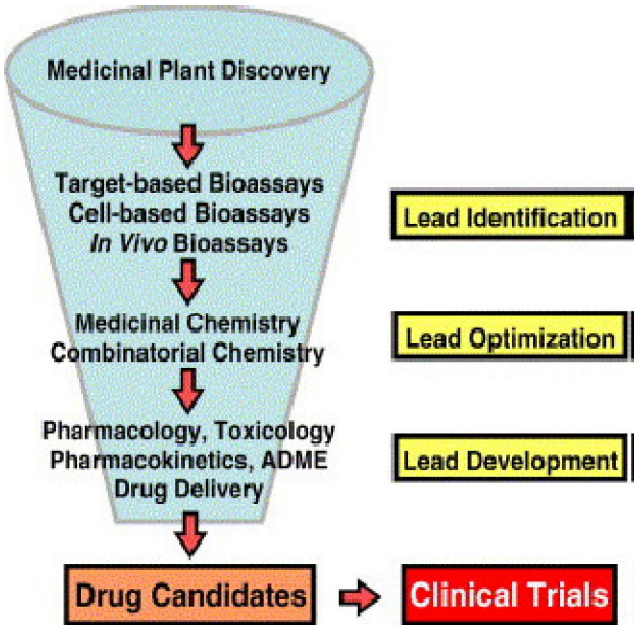


Figure 1.16: Schematic representation of a typical medicinal plant drug discovery process and development.

Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. Therefore, many pharmaceutical companies have eliminated or scaled down their natural product research. Recently, there has been a rekindling of interest in 'rediscovering natural products'. As stated by one authority "We would not have the top-selling drug class today, the statins; the whole field of angiotensin antagonists and angiotensin-converting enzyme inhibitors; the whole area of immunosuppressives, nor most of the anticancer and antibacterial drugs. Imagine all of these drugs not being available to physicians or patients today". It is clear that nature has played and will continue to play, a vital role in the drug discovery process.

1.7 Synthesis and Role of Plant Metabolites

In plants, as a result of metabolic processes, many different kinds and types of organic compounds or metabolites are produced. These metabolites are grouped into primary and secondary metabolites.

1.7.1 Primary Metabolites

The primary metabolites like chlorophyll, amino acids, nucleotides, simple carbohydrates or membrane lipids, play recognised roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation.

1.7.2 Secondary Metabolites

The secondary metabolites also differ from primary metabolites in having a restricted distribution in the plant kingdom. That is, particular secondary metabolites are often found in only one plant species or a taxonomically related group of species, whereas the basic primary metabolites are found throughout the plant kingdom. During the past few decades, experimental and circumstantial evidence has made it clear that many secondary metabolites do indeed have functions that are vital for the fitness of a plant producing them. The main roles are:

- Defence against herbivores (insects, vertebrates)
- Defence against fungi and bacteria
- Defence against viruses
- Defence against other plants competing for light, water and nutrients
- Signal compounds to attract pollinating and seed dispersing animals
- Signals for communication between plants and symbiotic microorganisms (e.g. N-fixing Rhizobia or mycorrhizal fungi)
- Protection against UV-light or other physical stress.

Plant secondary metabolites can be grouped into three chemically distinct classes:

- terpenes,
- phenolics and
- nitrogen containing compounds. (Adelekan, 2009).

1.8 Antimicrobial Activity of Plants

Turmeric Ginger Clove

Figure 1.17: Some Plants having Antimicrobial Activity.

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural. In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years.

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections.

In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to 8 continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases. Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density

lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics.

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains. The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs.There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials.

1.9 Role of Plants as Antioxidants in Disease Prevention

Generally, reactive oxygen species (ROS), reactive nitrogen species (RNS) and free radicals in the body are generated through exogenous (radiation, cigarette smoke,atmospheric pollutants, toxic chemicals, over nutrition, changing food habits, etc. intake of fruit and vegetables, overweight, obesity, and physical inactivity.

Car-diovascular diseases are mainly referred to as congestive heart failure, systolichypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, arrhythmia, etc. numerous medicinal plants, such as *Digitalis lanata,D.purpurea,Apocynum cannabinum,Calotropis procera,Carissa spectabilis,Nerium oleander,Urginea rubra*, etc. contain potent cardioactive glycosides and have positive inotropic actions on the heart.The drug digitoxin,digoxin reported from Digitalis lanata and D. purpurea has been used in the treatment of congestive heart failure for many decades. Besides, increased intake of antioxidant, extracellular and long-lived proteins,such as elastin, laminin, collagen, etc., are reported to prevent cardiovascular diseases.

Exogenous antioxidant from natural compounds , i.e., curcumin, bai-calen, and resveratol prevent arthrosclerosis formation by exhibiting radicalscavenging effects.

A number of flavonoids, including quercetin, morin, gos-sypetin, chrystin, myrecetin, rutin, catechin, and its derivatives and some oligo-meric proanthocyanidins are reported to inhibit the oxidation of LDL in invitro studies.

Some of the flavonoids obtained from leaves of Morus alba, such as quercetin 3-(6-malonyglucoside) are reported to attenuate the arthrosclerosis lesion development in LDL receptor in deficient mice through enhancement of LDL resistance to oxidation modification. (Bhatt, 2016).

1.10 Medicinal Plants as The Best Choice for Cancer Treatment

- The chemical components of medicinal plants mainly possess antioxidant properties that contribute to their anticancer potential. Flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins are the major classes of bioactive constituents responsible for the antioxidant action.
- The great potential of plant-based compounds for the treatment and prevention of cancer is attributed to their safety, low cost, and oral bioavailability. However, a few plant-based compounds induce some side effects. These side effects can be overcome by dose-dependent administration and usage, and do not in any case make them unsuitable for phytochemical research.
- The already available expensive conventional therapies for cancer like chemotherapy and radiotherapy have a number of side effects such as myelosuppression and neurological, cardiac, pulmonary, and renal toxicity, which pose serious harm to the quality of life. Therefore, there is a need to develop treatment options that include more potent and less toxic anticancer drugs as compared to existing drugs.
- The market statistics mark the availability of approximately 60% plant-based anticancer drugs.Medicinal plants constitute a common alternative to cancer treatment in many countries of the world.
- Cytotoxic screening of a number of plants has been done to correlate their anticancer activity and further expand their scope for drug development.

- Owing to potential benefits of plant based drugs for cancer treatment, their use is increasingly growing from 10% to 40% across the globe; specifically, on the Asian continent, it has reached 50%. Anticancer benefits associated with natural plant derivatives demand extensive scientific screening and clinical experimentations for the development of improved drugs. (Raina et al.,2014).

1.11 Herbal Medicine: Advantages and Disadvantages

There are numerous advantages and disadvantages of herbal medicine. Anyone considering using herbal medicine to treat health conditions should speak with a qualified health professional.

Advantages

There are a number advantages associated with using herbal medicines as opposed to pharmaceutical products. Examples include the following:

- **Reduced risk of side effects:** Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.
- **Effectives with chronic conditions:** Herbal medicines tend to be more effective for long-standing health complaints that don't respond well to traditional medicine. One example is the herbs and alternative remedies used to treat arthritis. Vioxx, a well-known prescription drug used to treat arthritis, was recalled due to increased risk of cardiovascular complications. Alternative treatments for arthritis, on the other hand, have few side effects. Such treatments include dietary changes like adding simple herbs, eliminating vegetables from the nightshade family and reducing white sugar consumption.
- **Lower cost:** Another advantage to herbal medicine is cost. Herbs cost much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.
- **Widespread availability:** Yet another advantage of herbal medicines are their availability. Herbs are available without a prescription. You can grow some simple herbs,

such as peppermint and chamomile, at home. In some remote parts of the world, herbs may be the only treatment available to the majority of people.

Disadvantages

Herbs are not without disadvantages, and herbal medicine is not appropriate in all situations. These are a few of the disadvantages to consider:

- **Inappropriate for many conditions:** Modern medicine treats sudden and serious illnesses and accidents much more effectively than herbal or alternative treatments. An herbalist would not be able to treat serious trauma, such as a broken leg, nor would he be able to heal an appendicitis or a heart attack as effectively as a conventional doctor using modern diagnostic tests, surgery, and drugs.
- **Lack of dosage instructions:** Another disadvantage of herbal medicine is the very real risks of doing yourself harm through self-dosing with herbs. While you can argue that the same thing can happen with medications, such as accidentally overdosing on cold remedies, many herbs do not come with instructions or package inserts. There's a very real risk of overdose.
- **Poison risk associated with wild herbs:** Harvesting herbs in the wild is risky, if not foolhardy, yet some people try to identify and pick wild herbs. They run a very real risk of poisoning themselves if they don't correctly identify the herb, or if they use the wrong part of the plant.
- **Medication interactions:** Herbal treatments can interact with medications. Nearly all herbs come with some warning, and many, like the herbs used for anxiety such as Valerian and St. John's Wort, can interact with prescription medication like antidepressants. It's important to discuss your medications and herbal supplements with your doctor to avoid dangerous interactions.
- **Lack of regulation:** Because herbal products are not tightly regulated, consumers also run the risk of buying inferior quality herbs. The quality of herbal products may vary among batches, brands or manufacturers. This can make it much more difficult to prescribe the proper dose of an herb.(Roberts, K.).

1.12 *Ficus hispida* Plant

Scientific classification

Kingdom: Plantae

Division: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *F. hispida*

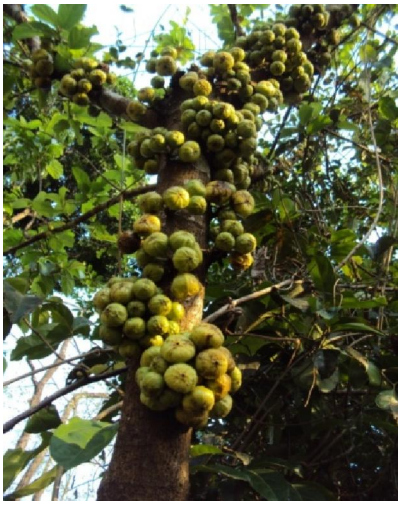


Figure 1.18: *Ficus hispida* plant

1.12.1 Local name:

Hindi name (Kathumar, Kathgular), English name (Hairy Fig, devil fig, opposite leaved fig), Arabic name (Tin basin), Assamese name (Khoskadumar), Bengali name (Kakadumbar). (Prashanth, 2017).

1.12.2 Description of the Plant:

Dumur or Hairy fig (*Ficus hispida*) is a medium-sized tree from ficus genus with branches. The hairy-leaved tree is native to Bangladesh, as well as South and Southeast Asia and New Guinea, Australia and Andaman island. The altitudinal range of the species is from sea level to about 800 m. Figs are eaten as vegetables in Bangladesh and India.

In parts of Cape York Peninsula, the figs provide a good supply of food for feral pigs. As the figs are produced down to ground level, the pigs can easily commandeer the lower part of the crop.

Several varieties have been named, based on fruit size and colour; but these vary too continuously to justify the subdivisions. Parts of the tree have been used in folk medicine to treat such varied complaints as stomach-ache, boils, warts, fever, diarrhoea, and in aiding parturition.

In some Asian countries the timber is used to make furniture and packing cases.The fertilizing wasp is a member of the Ceratosolen genus. (Some Magnetic Island Plants, 2016).

1.12.3 Morphology:

Stem

Not a strangling fig. Bark exudate pale brown or turning pink and then pale brown on exposure.

Leaves

Figure 1.19: Leaves of *Ficus hispida*

Leaf blades about 15-35 x 6-20 cm, rough and sandpapery on both the upper and lower surfaces. Petiole and twigs produce a watery milky yellow exudate. Flat glands usually visible on the underside of the leaf blade in the forks of the lateral veins and the midrib. Oil dots visible with a lens. Stipules about 0.6-1 cm long, slightly hairy and tapering to a point at the apex.

Flowers

Perianth entire, without any lobes. Male flowers produced around the ostiole. Style hairy on the upper half, stigma swollen, minutely papillose. Bracts at the base of the fig, three. Lateral bracts usually present on the outside of the fig body.

Fruit

Figs pedunculate, depressed globular to almost discoid, about 15-30 x 25-35 mm. Orifice closed by interlocking apical and internal bracts.

Figure 1.20: Fruits of *Ficus hispida*

Seedlings

Cotyledons orbicular, about 2-3 mm diam. At the tenth leaf stage: leaf blade ovate to obovate, apex acuminate, base obtuse, margin crenate, teeth mainly along upper 2/3 of the leaf blade; both the upper and lower surfaces scabrous; oil dots small, numerous, visible with a lens from the underside of the leaf blade; stipules sheathing the terminal bud, narrowly triangular, persistent, midrib hairy. (Australian Tropical Rainforest Plants, 2010).

1.12.4 Chemical Composition:

The fruit and the bark of the plant contain beta- sitosterol, beta- amyrin, n- triacontanyl acetate, gluacol acetate, hispidin, bergapten and psoralen. Bark also contains tannin, glucoside, wax & saponin Leaves contain oleanolic acid and phenanthroindolizidine alkaloids. Root contains leucocyanin.

1.12.5 Medicinal Uses:

- The fruit and bark of the tree is used for inducing purgation and emesis to remove excess pitta dosha from the body.
- Decoction from the bark of Kakodumbara is given in a dose of 40-50 ml to treat fever.
- The ripened fruit of Kakodumbara provides strength and nourishment to the body.
- The powder of the root bark of *Ficus hispida* is applied over the area affected with eczema
- The latex obtained from the fruit is applied directly over the fresh wound for quick healing and over the area having ring worm for treatment.

- Decoction prepared from the fruit or bark of the *Ficus hispida* is given in a dose of 50 ml to treat jaundice, piles and distention of abdomen.
- The fruit juice of ripened *Ficus hispida* along with honey acts as anti- hemorrhagic.
- Consuming the ripened fruit is a good source for increasing the breast milk in lactating women.
- Root powder in a dose of 3-5 gm is given with buttermilk to increase the appetite and improve digestion.
- In case of vitiligo, latex of unripe fruit of *Ficus hispida* is given with jiggery to induce purgation as part of treatment.
- To treat diarrhea, decoction of bark of *Ficus hispida* along with tender leaf of mango is prepared and given in a dose of 40-50 ml.
- In cases of excessive bleeding during menstruating, the fruit of Kakodumbara along with honey is given to provide strength to body and control bleeding.
- In case of dog bite, intake of Kakodumbara root mixed with Dhattura seeds (*Nux vomica)* along with rice water destroys.
- To treat diabetic ulcers, the latex of unripe fruit of Kakodumbara is applied over the ulcers.
- To treat cervical spondylitis, latex of the fruit is mixed with Hingu (Asafoetida) and root of Kapikachu (*Mucuna prurita*) powder is used as nasal snuff. (Prashanth, 2017).

CHAPTER TWO
LITERATURE REVIEW

Literature Review

2.1 *Ficus hispida* Linn.: A Review of its Pharmacognostic and Ethnomedicinal Properties

- **Hypoglycemic activity**

Ghosh *et al*, have successfully demonstrated the hypoglycemic activity of FH bark in diabetic albino rats. They reported that water soluble portion of ethanolic extract of the bark showed significant reduction of blood glucose level, increase in the uptake of glucose and increase in the glycogen content of liver, skeletal muscle and cardiac muscle. They also revealed the interaction of the constituent of the extract with insulin on concomitant administration, but the compound involved is not yet established.

- **Cardioprotective effect**

Shanmugarajan *et al*, evaluated the cardioprotective effect of FH leaf extract on cyclophosphamide mediated myocardial injury due to oxidative stress in rat heart. This study showed that the extract exhibited a significant inhibition of lipid peroxidation and increased the level of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and reduced the glutathione activity in heart tissue provoked by cyclophosphamide.

- **Antidiarrheal activity**

Mandal *et al*, prepared the extracts from the leaves of FH and assessed the antidiarrheal activity in rats against castor-oil induced diarrhea and PEG_2-induced interpooling in rats. The methanolic extract of leaves showed a significant and dose-dependent antidiarrheal activity and also reduced the propulsion of charcoal meal through the gastrointestinal tract when administered orally. They also established the dose of the extract and found that at the level of 600 mg/kg, the response was equivalent to that produced by 5 mg/kg of diphenoxylate.

- **Antiulcerogenic effect**

Sivaraman *et al*, conducted a study on the methanolic extract of FH root in aspirin ulcerated rats and showed that a dose of 200 and 400 mg/kg substantially decreased the incidence of ulcers, enhanced the healing of ulcers and significantly reduced free and total acidity.

- **Sedative and anticonvulsant effects**

Dhanasekaran *et al*, administered the methanolic extract of leaves at a dose of 200 and 400 mg/kg and investigated phenobarbitone induced sleeping time and hole-board exploratory behavior for sedation test, and strychnine, picrotoxin, and phenylenetetrazole-induced convulsion in swiss albino mice. The result proved that there was a significant and dose-dependent reduction in the onset and prolongation of sleep duration elicited by phenobarbitone. In addition to this, suppression of exploratory action was reported. Moreover, complete inhibition of seizures induced due to picrotoxin and strychnine was seen at a dose of 400 mg/kg, along with significant prolongation of both clonic and tonic seizures.

- **Neuroprotective effects**

Sivaraman *et al*, established the neuroprotective effect of methanolic extract of leaves of FH on b-amyloid induced cognitive deficits and oxidative stress in mice. Study showed that the extract impairs the cognitive behavior and memory deficit and suppresses the increased level of thiobarbituric acid reactive species in brain. Additionally, increased activities of antioxidant enzymes like glutathione peroxidase, glutathione reductase and superoxide dismutase were also seen in the study. These activities may be helpful in the holistic treatment of Alzheimer′s disease and other age-related memory impairments.

- **Hepatoprotective effect**

Shanmugarajan *et al*, prepared the methanolic extract of leaves of FH and successfully confirmed the hepatoprotective effect on cyclophosphamide mediated oxidative liver injury in Wistar rat. In another study (performed by the same group), methanolic leaf extract was explored against azathioprin elicited liver injury in Wistar rat liver. Mandal *et al*, extracted leaves of FH and investigated for hepatoprotection in rats by inducing acute liver damage by paracetamol. The results were found to be comparable to that of a standard Liv-52 formulation, a known hepatoprotective formulation

- **Antineoplastic activity**

Pratumvinit *et al*, depicted the antineoplastic effect of FH stem successively extracted with crude ethanol; water, methanol; water, methanol and ethyl acetate fractions against SKBR3, MDA-MB435, MCF7 and T47D human breast cancer cell lines in vitro. Only the ethanolic fraction was found to have anti-neoplastic activity against T47D cells.

- **Anti-inflammatory and antipyretic**

Vishnoi *et al*, showed significant activity in the carraggenan induced paw edema in albino rat model, reducing inflammation by approximately 64.07% compared to only 45.13% with the standard, diclofenac sodium. (Ali et al., 2011).

2.2 Effect of *Ficus hispida* L. on Normal and Dexamethasone Suppressed Wound Healing

Ethanolic extract of roots of *Ficus hispida* was investigated in normal and dexamethasone depressed healing conditions, using incision, excision and dead space wound models in albino rats.

The root extract of *Ficus hispida* has shown the maximum breaking strength compared to control group. The rate of epithelialization and wound contraction in excision model was better as compared to control groups. There was significant increase in granulation tissue weight and hydroxyproline content in dead space model compared to control group. The antihealing effect of dexamethasone was also reverted by the administration of ethanolic extract of *Ficus hispida* in all the wound models .

The results indicated that the root extract of *Ficus hispida* has a significant wound healing activity and also promotes healing in dexamethasone depressed healing conditions. (Panchal et al., 2011).

2.3 *Ficus hispida* Bark Extract Prevents Nociception, Inflammation, and CNS Stimulation in Experimental Animal Model

Ficus hispida is traditionally used in the ailment of pain, inflammation, and neurological disorders. The present study set out to evaluate the in vivo antinociceptive, anti-inflammatory, and sedative activity of the ethanol extract of Ficus hispida bark (EFHB).

Methods: The antinociceptive activity of EFHB was evaluated by using acetic acid induced writhing, formalin, hot plate, and tail immersion methods in Swiss albino mice. Its anti-inflammatory activity was assessed by using carrageenan and histamine induced rat paw oedema test in Wister rats. The central stimulating activity was studied by using pentobarbital induced hypnosis, hole cross, and open field tests in Swiss albino mice.

Results: EFHB demonstrated antinociceptive activity both centrally and peripherally. It showed 62.24% of writhing inhibition. It significantly inhibited licking responses in early (59.29%) and late phase (71.61%). It increased the reaction time to the thermal stimulus in both hot plate and tail immersion. It inhibited the inflammation to the extent of 59.49%. A substantial increase in duration of sleep up to 60.80 min and decrease of locomotion up to 21.70 at 400 mg/kg were also observed. Conclusion. We found significant dose dependent antinociceptive, anti-inflammatory, and sedative properties of EFHB in experimental animal models. (Siraj et al.,2017).

2.4 In-vitro Antineoplastic Effect of *Ficus hispida* L. Plant against Breast Cancer Cell Lines

Stems of *Ficus hispida* L. have long been prescribed as one of the constituents in various Thai traditional remedies for cancer therapy.

In the present study, crude ethanol extract and its sequential fractions from *F. hispida* L.: water, methanol: water, methanol and ethyl acetate fraction were tested in vitro against SKBR3, MDA-MB435, MCF7 and T47D human breast cancer cell lines.

The results have shown that the methanol extract exhibited antineoplastic activity against T47D cells. The cytotoxic activity was further examined by MTT assay with more dilution, colony forming assay and cell cycle analysis. The IC50 of this extract against T47D cell was 110.3 +/- 9.63 g/mL by MTT assay and colony forming assay confirmed the cell growth inhibition in a dose-dependent manner. Cell cycle analysis demonstrated a rising of apoptotic cell population in

herbal treated cells. Therefore, *F. hispida* L. used in traditional medicine may provide some benefits in the treatment of breast cancer. (Pratumvinit et al., 2009).

2.5 Pharmacognostic and Phytochemical Investigation on Leaves of *Ficus hispida*

Ficus hispida (Syn: *Ficus oppositifolia* Roxb; Family: Moraceae) commonly known as devil fig, hairy fig is grows in Tropical and Subtropical regions of India, used for variety of purpose in traditional medicine.

The usefulness of this plant is described in many folk books including Ayurveda and is scientifically evidenced, and different biologically active phytoconstituents were isolated from plant. But no reports are available on morph anatomy, and phytochemical studies, hence present attempt was undertaken to investigate the microscopically and preliminary phytochemical and Physico-chemical studies on the leaves of *Ficus hispida.*

The study reveals the leaves are simple, opposite, decussate, caducous. The transverse section of the leaves shows presence of epidermis, sponge parenchyma, bicollateral vascular bundles, nonglandular, glandular trichome and spiral vessels. The powder microscopy revealed the presence of anomocytic stomata, glandular trichome, covering trichome and prismatic calcium oxalate crystals.

Physicochemical parameters like ash value, extractive value and phytochemical screening with different reagents showed the presence of -

- fluorescence compounds,
- steroids,
- triterpenoids,
- phenols,
- tannins and
- flavonoids. (Paarakh et al., 2011).

2.6 In-vitro Anti-oxidant & Antimicrobial Study of *Ficus hispida*

Ficus hispida L. belongs to the Moraceae family and is used by the maaiba trible (indigenous medicine - man of Manipur, India) as an indigenous traditional medicine. Present study deals with the successive extraction of the aerial parts of *Ficus hispida* and in-vitro screening of anti-oxidant and anti-microbial activity.

The phytochemical screening of the methanol extract of *Ficus hispida* shows the presence of secondary metabolite groups like –

- alkaloid,
- phenolic compounds,
- flavonoid,
- glycosides,
- protein etc.

Phenolic compounds are commonly found in both edible and nonedible plants and are responsible for various medicinal activities of plants, so our study is based on determining antioxidant activity and anti-microbial activity.

Beside these, we also measured the total flavonoid and total phenolic content of the respective sample to understand the effect of polyphenolic compound on different pathophysiological state associated with high free radical production.

The in-vitro investigation proves the efficiency of this plant in various diseases states. (Mondal et al., 2015)

2.7 Evaluation of Antimicrobial Activities of Methanolic Extract And Fractions of *Ficus hispida* L. Fruits

The present study was carried out to investigate the methanolic extract of *Ficus hispida* (Bengali name- Kakdumur Family-Moraceae) fruits and it's fractions (chloroform, ethyl acetate and aqueous fractions) for their in vitro antimicrobial activities. The test was carried out against six pathogenic bacteria using agar disc diffusion method.

The extract and it's fractions exhibited reasonable antibacterial activities against three gram positive (*Bacillus cereus, Staphylococcus aureus, Agrobacterium*) species and three gram negative (*Escherichia coli, Shigella dysenteriae, Shigella sonnei*) pathogenic bacteria.

Ethyl acetate fraction (EAF) showed highest zone of inhibition (20.5 and 28 mm in diameter) against E. coli at a concentration of 200 and 400 µg/disc, respectively. The activity of crude methanolic extract (CME) was higher (20 nm in diameter) than EAF against gram positive bacteria. The chloroform fraction (CHF) did not show any activity against both gram positive and gram negative bacteria. Our present study suggests that active antimicrobial agents present in the extract of *Ficus hispida* fruits may have potential for the treatment of bacterial infection. (Hossain et al., 2014).

2.8 Antinociceptive and Neuropharmacological Activities of Ethanolic Extract of the Fruits of *Ficus hispida* Linn.

The crude ethanolic extract of the fruits of *Ficus hispida* Linn. (Family: Moraceae) growing in southeast part of Bangladesh has been evaluated for its possible antinociceptive and neuropharmacological properties.

The ethanolic extract of the fruits of *F. hispida* exhibited statistically significant ($p < 0.001$) writhing inhibition in acetic acid-induced writhing model in white albino mice (Swiss-webstar strain). The crude extract produced 30.41% inhibition of writhing at the dose of 250 mg/kg body weight and 62.84% inhibition of writhing at the dose of 500 mg/kg body weight, while the standard drug diclofenac inhibition was found to produce 75.68% at a dose of 25 mg/kg body weight. The extract of *F. hispida* fruits also potentiated the pentobarbital induced sleeping time in mice, decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test. Acute toxicity test showed that the plant might be safe for pharmacological uses.

Therefore, the obtained results tend to suggest the antinociceptive and neuropharmacological activities of the ethanolic extract of the fruits of *F. hispida* and thus, provide the scientific basis for the traditional uses of this plant part as a remedy for pain and depression. The obtained

results provide a support for the use of this plant in traditional medicine and its further investigation. (Howlader et al., 2012).

2.9 Thrombolytic Activity and Antimicrobial Properties of *Ficus hispida*

In this present study, the various plant parts of *Ficus hispida* were subjected to thrombolytic and antimicrobial activities. The thrombolytic activities were assessed by using human blood samples and the results were compared with standard streptokinase (SK).

In this study, the methanol soluble fraction (MSF) exhibited highest thrombolytic activity (50.12±1.91). However, significant thrombolytic activity was demonstrated by the crude ethanol extract (CEE) and n-hexane soluble fraction (HSF) of *F. hispida* (21.74±0.69) and (42.22±1.42) respectively.

On the other hand, the n- hexane soluble fraction (HSF) and methanol soluble fraction (MSF) of ethanol extract revealed moderate antibacterial activity against some microorganisms used in the screening. (Shahriar et al., 2013).

2.10 Hypoglycemic Activity of *Ficus hispida* (bark) in Normal & Diabetic Albino Rats

Objective: To find out the hypoglycemic activity of *Ficus hispida* Linn. (bark) in normal and diabetic albino rats and to evaluate its probable mechanism of hypoglycemic activity if any.

Material and Methods: Albino rats were divided into groups (n=6) receiving different treatments consisting of vehicle, water-soluble portion of the ethanol extract of *Ficus hispida* bark (FH) (1.25 g/ kg) and standard antidiabetic drugs, glibenclamide (0.5 mg/kg) and 0.24 units of insulin (0.62 ml of 0.40 units/ml). Blood glucose was estimated by the glucose oxidase method in both normal and alloxan-induced diabetic rats before and 2 h after the administration of drugs. To find out the probable mechanism of action of FH as a hypoglycemic agent,

i) the glycogen content of the liver, skeletal muscle and cardiac muscle, and
ii) glucose uptake by isolated rat hemi-diaphragm were estimated.

Results: FH showed significant reduction of blood glucose level both in the normal ($P<0.01$) and diabetic ($P<0.001$) rats. However, the reduction in the blood glucose level was less than that of thestandard drug, glibenclamide. FH also increased the uptake of glucose by rat hemi-diaphragm significantly ($P<0.001$). There was a significant increase in the glycogen content of the liver ($P<0.05$), skeletal muscle ($P<0.01$) and cardiac muscle ($P<0.001$). The amount of glycogen present in the cardiac muscle was more than the glycogen present in the skeletal muscle and liver.

Conclusion: FH has significant hypoglycemic activity. Increased glycogenesis and enhanced peripheral uptake of glucose are the probable mechanisms involved in its hypoglycemic activity. (Ghosh et al., 2004).

2.11 Anti-ulcerogenic Evaluation of Root Extract of *Ficus hispida* Linn. in Aspirin Ulcerated Rats

The present study was designed to investigate the anti-ulcer efficacy of methanolic root extract of the *Ficus hispida* Linn. (FH), which was known to possess various therapeutic properties. The reason for the study was that the known non-steroidal anti-inflammatory drugs (NSAIDs) were full of side effects especially ulceration causes Gastric ulceration an economic loss and a source of welfare concern worldwide. There are 350,000 to 500,000 new cases per year and more than one million are ulcer-related hospitalizations.

We found that FH decreased the incidence of ulcers and also enhanced the healing of ulcers. Methanolic extract of FH at doses 200 and 400 mg/kg was found to be effective by 63.8 and 68.44% respectively in aspirin (ASP) induced ulcer model and significantly reduced free and total acidity. It was observed that anti-ulcer effect of FH might be due to its cytoprotective effect rather than antisecretory activity.

Conclusively, FH was found to possess potent anti-ulcerogenic as well as ulcer healing properties and could act as a potent therapeutic agent against peptic ulcer disease. (Sivaraman et al., 2010).

CHAPTER THREE

METHODS AND MATERIALS

Methods and Materials

3.1 Collection and preparation of plant material

Plant sample of *Ficus hispida* collected from Munshigonj in March, 2017. Then proper identification of plant sample was done by an expert taxonomist. The plant was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried plant was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Extraction of the plant material

About 650 gm of the powdered material was taken in separate clean, round bottomed flask (5liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 390°C with a Heidolph rotary evaporation.

The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 25.18 gm respectively.

Figure 3.1: Drying of extract using rotary evaporator

3.3. Preparation of Mother Solution

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

3.4. Partition of Mother Solution

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

3.4.1. Partition with Pet-ether

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

3.4.2 Partition with Dichloromethane

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

3.4.3 Partition with Ethyl acetate

To the mother solution that left after washing with Pet-ether, and Dichloromethane, 16 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with ethyl acetate. The process was repeated thrice (100 ml X 3). The ethyl acetate fraction was then air dried for solid residue.

3.4.4 Partition with Aqueous Fraction

After partitioning the mother solution with Pet-ether, Dichloromethane and Ethyl acetate, 20 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with aqueous fraction. The process was repeated thrice (100 ml X 3). The aqueous fraction was then air dried for solid residue.

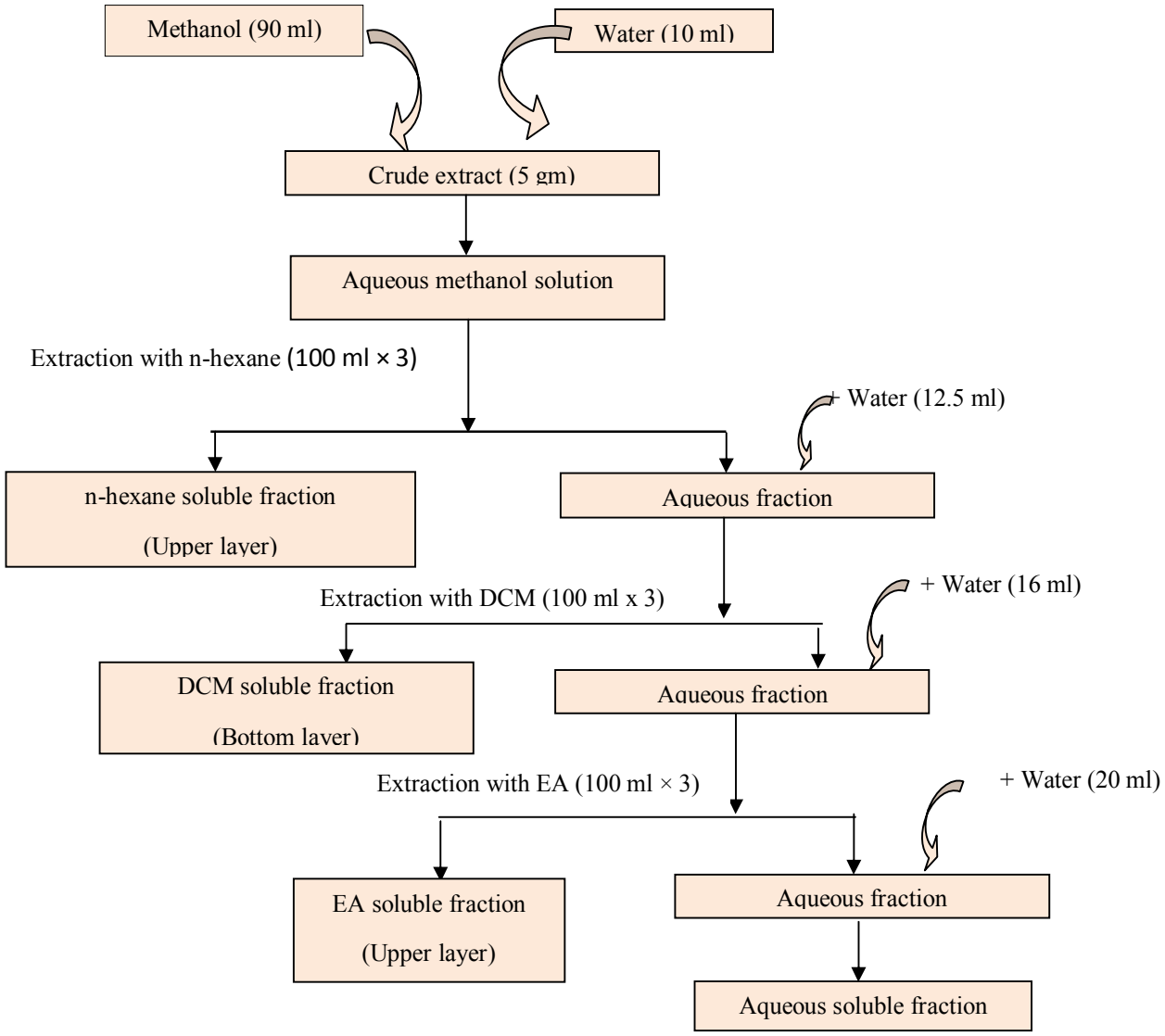


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Ficus hispida.*

3.4.5 Collection of DCM Fraction

After partitioning the mother solution with the four different solvents the DCM fraction of them were collected and air dried. This DCM fraction was further investigated for different pharmacological properties such as Antioxidant and Cytotoxic.

3.5 Brine Shrimp Lethality Bioassay

3.5.1 Principle

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

3.5.2 Apparatus & Reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

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

Lamp to attract shrimps	Test samples

3.5.3 Procedure

3.5.3.1 Preparation of Sea Water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 38 gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000 ml by distilled water in a 1000 ml beaker for *Artemia salina* hatching. 1-2 drops of 1 N NaOH or 1 N HCl solution was added with a dropper for obtaining the pH 8.4 as sea water.

3.5.3.2 Hatching of Brine Shrimp

A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Then a dry preserved egg of *Artemia salina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemiasalina* were hatched at room temperature (25-30ºC) for 18-24 hours. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5 ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay (Niazi J. *etal*., 2009).

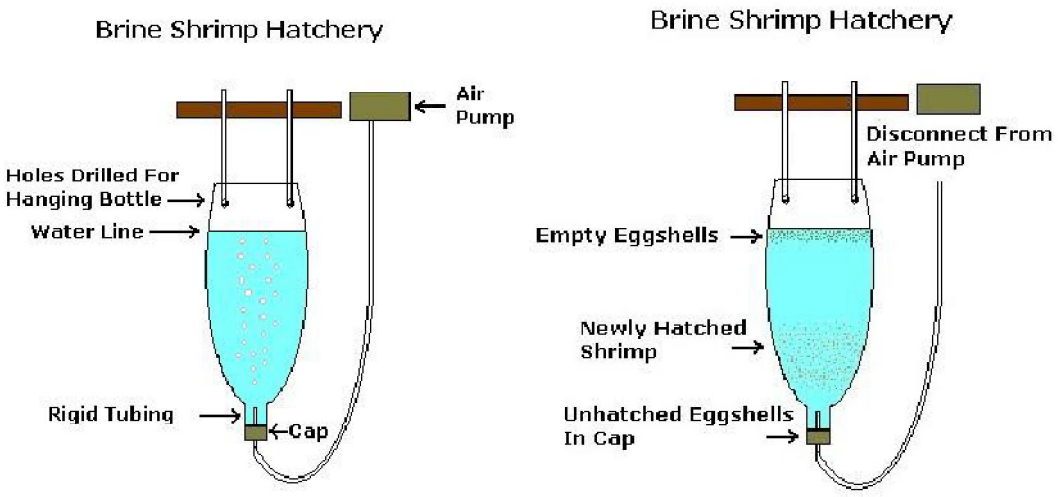


Figure 3.3: Brine shrimp Hatchery.

3.5.3.3 Preparation of Test Solutions

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

3.5.3.4 Preparation of the Test Samples of Experimental Plant

All the test samples of 4 mg were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions. Then 100 µl of solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100 µl DMSO was added to vial. Thus the concentrations of the obtained solution in each test tube were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml for 10 dilutions.

3.5.3.5 Preparation of the Positive Control Group

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 2000 µg/ml. From that stock solution serial dilutions are made using DMSO to get 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml. Then ten living brine shrimp nauplii in 5 ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.5.3.6 Preparation of the Negative Control Group

100 µl of DMSO was added to the pre-marked test tube containing 5 ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds (Goldstein *et al.*, 1974).

3.5.3.7 Counting of Nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration (Sleet RB and Brendel K, 1983).

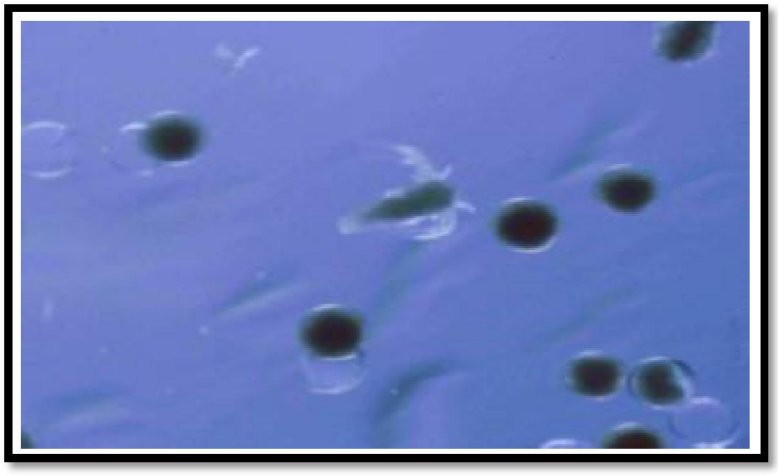


Figure 3.4: Counting of nauplii

3.6 Antioxidant Activity

3.6.1 Total Phenolic Content

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the antioxidative status occurrences of human diseases. In addition, antioxidant compounds which are responsible for such antioxidant activity could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidant effects have rarely been carried out. The purpose of this study was to evaluate extractives of *F. hispida* new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity.

3.6.1.1 Principle

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. In the alkaline condition phenols ionize completely.

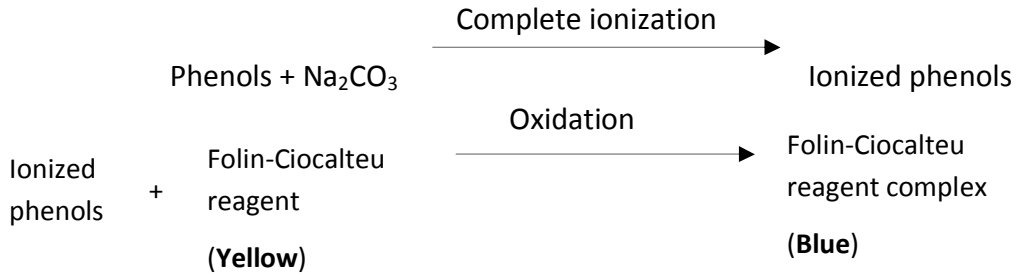
Table 3.2: Composition of 100 mg Folin-Ciocalteu Reagent

Composition of 100 mg Folin-Ciocalteu Reagent	
Water	57.5 ml
Sodium Tungstate Dihydrate	10.0 mg
Hydrochloric Acid (25%)	10.0 mg

Phosphoric Acid 85% solution in water	5.0 mg
Molybdic Acid Sodium Dihydrate	2.5 mg
Lithium Sulfate	15 mg

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain hetero-polyphosphotunstates - molybdates. Sequences of reversible oneor two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.

The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton et al., 1999).



3.6.1.2 Apparatus & Reagents

Table 3.3: Apparatus and reagents used for total phenolic content

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

Distilled water	Cuvette

3.6.1.3 Procedure

Standard curve preparation

Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120 µg/ml to 80 µg/ml. 5 ml of FCR (diluted 10 times with water) and 4 ml of Na_2CO_3 (7.5% w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765 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

2 mg of the *F. hispida* DCM fraction was taken and dissolved in 1 ml methanol to get a sample concentration of 2 mg/ml.

Determination of total phenol content

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

3.6.2 Total Flavonoid Content

3.6.2.1 Principle

Aluminium chloride ($AlCl_3$) colorimetric method is incorporated to determine the total flavonoid contents of the crude plant extract. The basic principle of the assay method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols of the crude extract. In addition aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A or B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorbance maximum at 510 nm. Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510 nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard. (Chang *et al.,* 2002)

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

3.6.2.2 Apparatus & Reagents

Table 3.4: Apparatus and reagents used for total flavonoid content

Aluminium chloride	Spatula
Methanol	Analytical balance
Quercetin	Pipette and pumper
Sodium hydroxide	Aqueous fraction
Sodium nitrite	Test tubes and beaker

3.6.2.3 Procedure

Preparation of 10% Aluminium Chloride ($AlCl_3$) Solution: 1gm of $AlCl_3$ was taken into a 10 ml of a volumetric flask and the volume was adjusted by distilled water.

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

Preparation of 5% (W/V) NaNO2 Solution: 0.5 gm of $NaNO_2$ was taken into a 10 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of Standard Solution: The stock solution was prepared by taking 10 mg of quercetin and dissolved into 50 ml of methanol. Concentration of this solution was 200 µg/ml of quercetin. The experimental concentrations were prepared from this stock solution.

Table 3.5: Preparation of standard solution

Concentration (µg/ml)	Solution taken from stock solution (ml)	Solution taken from stock solution (ml)	Final volume (ml)
0	0.0	5.0	5
4	0.1	4.9	5
8	0.2	4.8	5
12	0.3	4.7	5
16	0.4	4.6	5

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

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

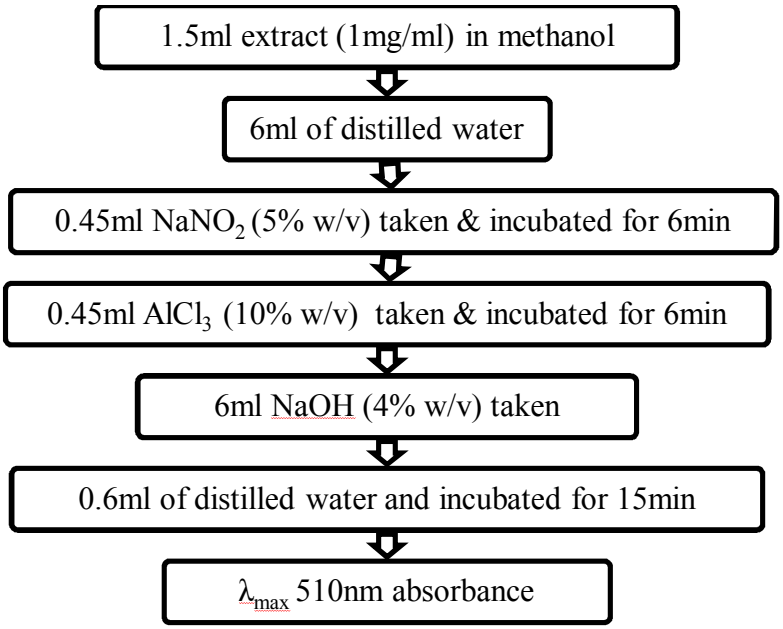


Figure 3.5: Schematic diagram of preparation of extract solution

Preparation of blank solution

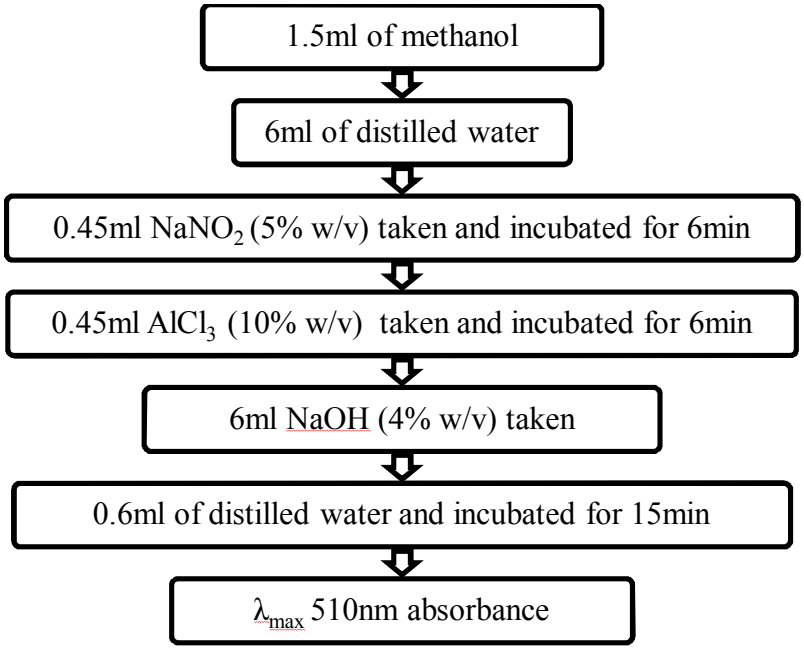


Figure 3.6: Schematic diagram of preparation of blank solution

CHAPTER FOUR
RESULTS AND DISCUSSION

Results and Discussion

4.1 Result of Brine Shrimp Lethality Bio-Assay

The ethyl acetate of the *Ficus hispida* extract was subjected to brine shrimp lethality bioassay. After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a Median Lethal Concentration (LC_{50}) value. LC_{50} represents the concentration of the standard and aqueous extract that produces death in half of the test subjects after a certain period. The percentage mortality at each concentration was determined using the following formula:

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

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

4.1.1 Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard.

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

Test tube no.	**Concentration (C) (µg/ml)**	**Log C**	**Number of Nauplii alive**	**Number of Nauplii dead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	6	4	40	35.23
2	200	2.301	0	10	100	
3	100	2.000	7	3	30	
4	50	1.699	4	6	60	

5	25	1.398	5	5	50	
6	12.5	1.097	5	5	50	
7	6.25	0.796	6	4	40	
8	3.125	0.495	3	7	70	
9	1.5625	0.194	10	0	0	
10	.078125	-0.107	0	10	10	

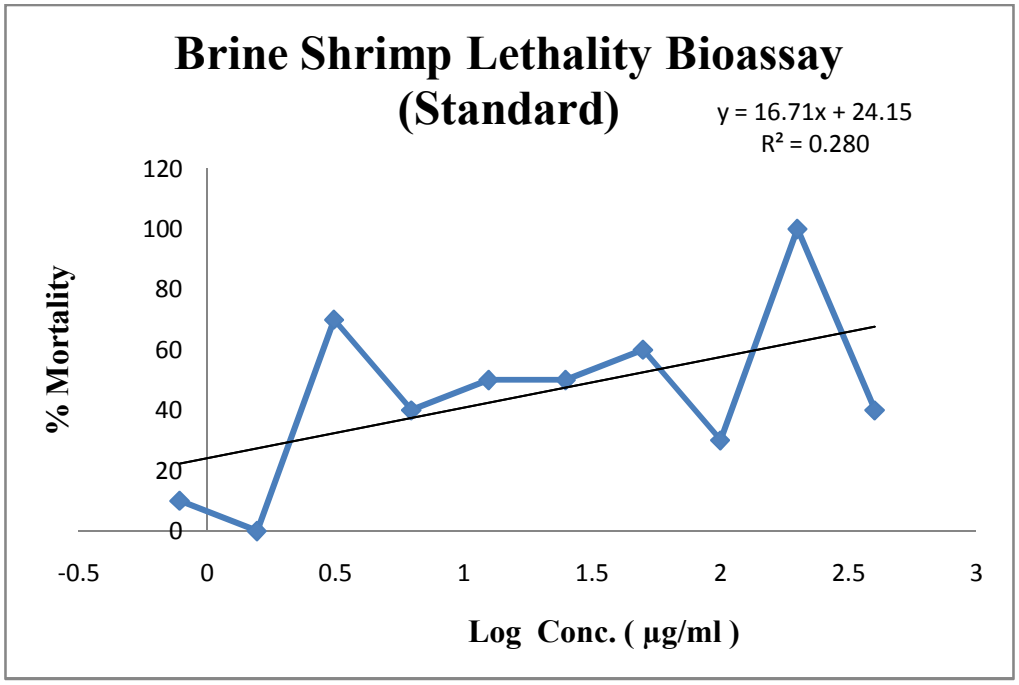


Figure 4.1: % Mortality and predicted regression line of Tamoxifen (standard).

4.1.2 Preparation of Ethyl Acetate (EA) Fraction Curve

Table 4.2: Results of the bioassay of Ethyl Acetate fraction (extract)

Test tube no.	**Concentration (C) (µg/ml)**	**Log C**	**Number of nauplii alive**	**Number of Nauplii dead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	0	10	100	1.79
2	200	2.301	3	7	70	
3	100	2.000	7	3	30	
4	50	1.699	4	6	60	
5	25	1.398	4	6	60	
6	12.5	1.097	5	5	50	
7	6.25	0.796	5	5	50	
8	3.125	0.495	6	4	40	
9	1.5625	0.194	5	5	50	
10	.078125	-0.107	3	7	70	

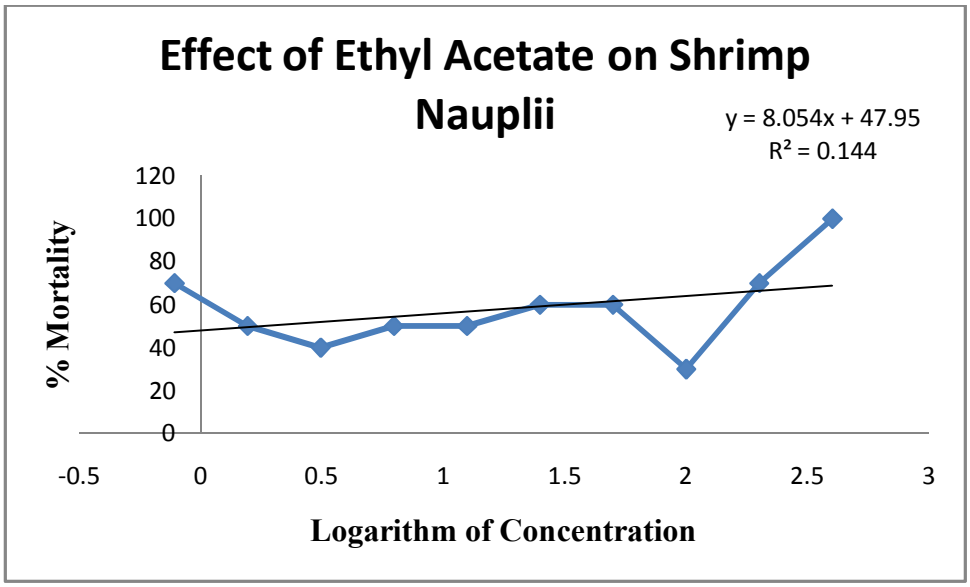


Figure 4.2: % Mortality and predicted regression line of Ethyl Acetate fraction (extract).

4.1.3 Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. There wasn't found any direct relationship between the lethality and concentration in both standard and EA fraction samples. In Table 4.1, 100% mortalities was found at concentration 200µg/ml which wasn't the highest concentration. In Table 4.2, 100% mortalities was found at the highest concentration but the degree of lethality didn't decrease with decrease in concentration. Again, 30% mortalities took place at the 100µg/ml, whereas 40% mortalities at concentration 6.25µg/ml and 3.125µg/ml as shown in Table 4.1 and Table 4.2 respectively.

Table 4.3: Cytotoxic activity of Tamoxifen and Ethyl Acetate fraction of *Ficus hispida* leaves

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml, 24hr)
Standard (Ascorbic Acid)	y = 16.71x + 24.15	0.280	35.23
Extract (Ethyl Acetate)	y =8.054x + 47.95	0.144	1.79

In this investigation, standard and EA fraction exhibited cytotoxic activities with the LC_{50} values 35.23µg/ml and 1.79µg/ml respectively as shown in Table 4.3. For EA fraction, LC_{50} value is

less than the standard which indicates that the extract has more potent activity than standard against brine shrimp nauplii.

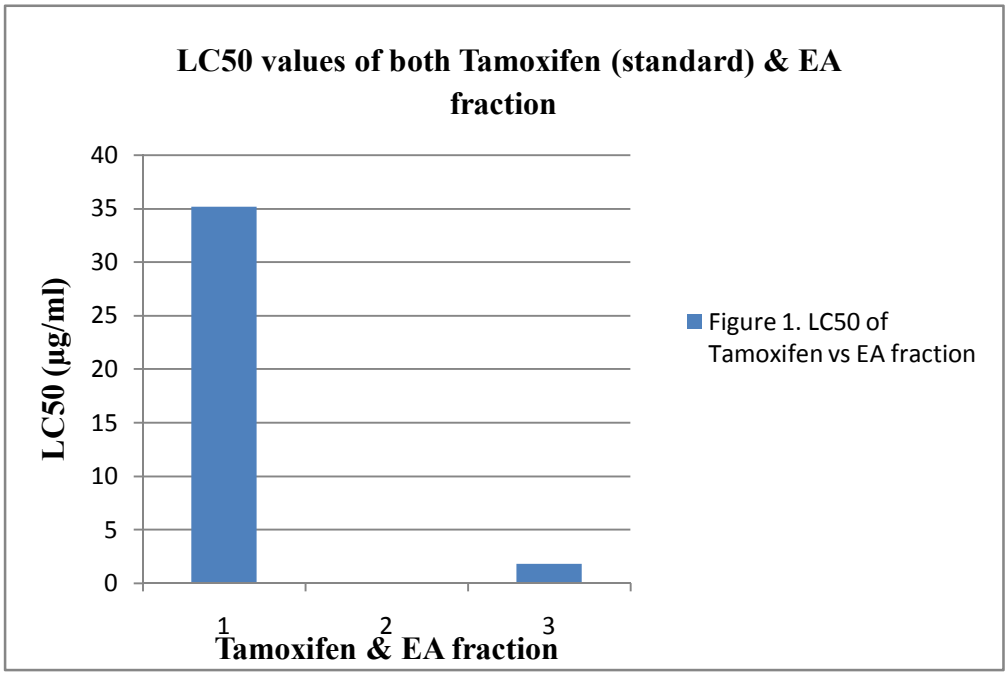


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for ethyl acetate fraction**,** the lethal concentration required to kill 50% of the sample population is lower than the standard. So the extract is more potent than Tamoxifen (standard) at lower concentration.

4.2 Result of Antioxidant Tests

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

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

4.2.1 Result of Total Phenolic Content

Ethyl Acetate fraction of *F. hispida* and the ethyl acetate of the methanol extract of *F. hispida* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard (Singleton et al., 1999).

4.2.1.1 Preparation of Standard Curve

Table 4.4: Total Phenolic content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 765 nm)**	**Regression line**	**R^2value**
80	2.642	y =0.024x+0.660	0.811
90	3.003		
100	2.962		
110	3.121		
120	3.806		

A non-linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.4. This curve was considered as a standard curve.

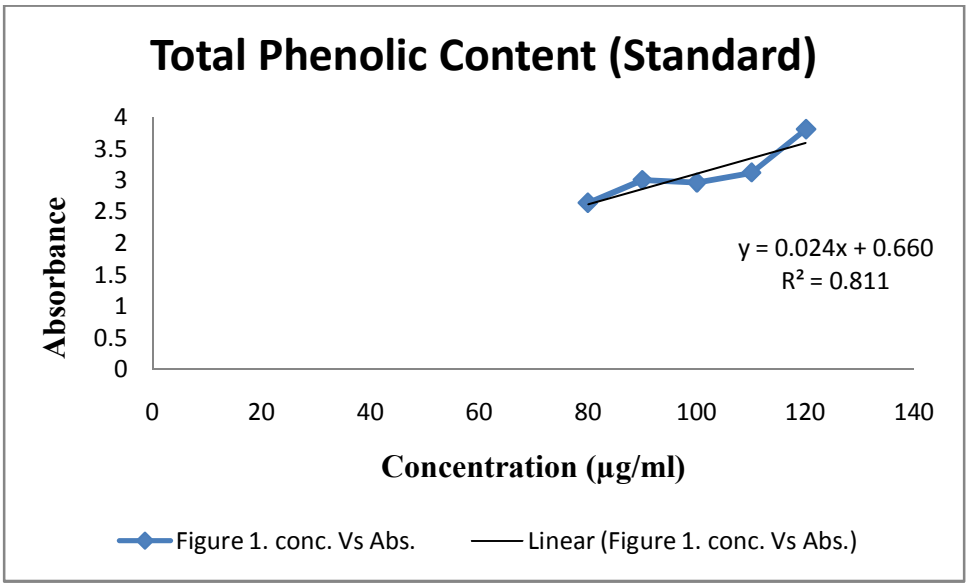


Figure 4.4: Graphical representation of Phenolic content of ascorbic acid

4.2.1.2 Total Phenolic content present in ethyl acetate of *Ficus hispida*

Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total phenolic content present in the extract is calculated and given in the table below.

Table 4.5: Total Phenolic content in ethyl acetate *of F. hispida*

Concentration (mg/ml)	Absorbance	mg AAE/g
2	4.00	139.167

4.2.1.3 Discussion

All the values of absorbances was found to be increased with increasing the concentrations except one. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the ethyl acetate is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 139.167 mg of AAE/gm of dried extract of phenol content was found in the EA of *F. hispida.*

4.2.2 Result of Total Flavonoid Content

The EA of *F. hispida* leaves were subjected to determine total flavonoid content. Quercetin was used as reference standard.

4.2.2.1 Preparation of Standard Curve

Table 4.6: Total flavonoid content of Quercetin.

Concentration (µg/ml)	Absorbance (At 420 nm)	Regression line	R^2 value
0	0	y = 0.053x-0.013	0.999
4	0.193		
8	0.422		
12	0.618		
16	0.834		

After absorbances were taken of different concentrations of quercetin ranging from 0µg/ml to 16µg/ml, a linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.5. This linear curve was considered as a standard curve.

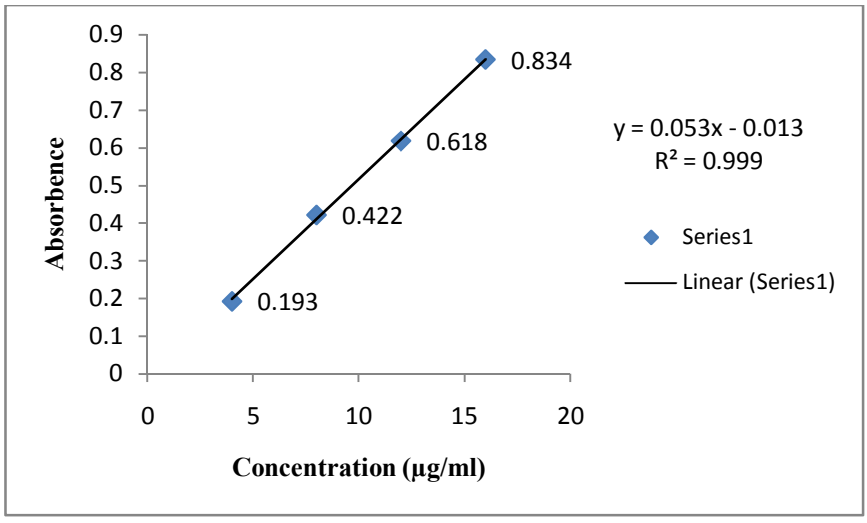


Figure 4.5: Graphical representation of assay of flavonoid content of quercetin

4.2.2.2 Total Flavonoid Content Present in Ethyl Acetate Extract

Based on the absorbance value of extract solution and using the regression line equation of the standard curve, the total flavonoid present in the extract is calculated and is given in Table 4.8.

Table 4.7: Total flavonoid content of EA fraction of *F. hispida* leaves extract.

Sample	Concentration (mg/ml)	Absorbance	Total flavonoid content (mg of quercetin equivalents /g of dried extract)
Ethyl acetate of *F. hispida*	1	0.503	9.73

4.2.2.3 Discussion

To determine the total flavonoid content of the test samples the standard curve was used. For 1mg/ml concentration of EA of *F. hispida* (leaves), 9.73 mg of quercetin equivalents/gm of dried extract of flavonoid content was found. So it can be said that, the extract contains very low antioxidative compounds.

CHAPTER FOUR

CONCLUSION

Conclusion

As the literature review suggests, the presence of several phytochemical compounds in EA fraction of *Ficus hispida,* makes the plant pharmacologically active.

LC_{50} value of *Ficus hispida* in aqueous fraction showed more cytotoxic activity than Tamoxifen. Since aqueous fraction of *Ficus hispida* exhibited potent cytotoxic activity, so it can be investigated for anticancer, pesticidal and antitumor properties in future.

Antioxidant property in aqueous extract of *Ficus hispida* was determined by Phenolic content assay and Flavonoid content. Phenolic content was 139.167 mg/gm and Flavonoid content was 9.73 mg/gm in aqueous extract of *Ficus hispida*. So EA fraction of *Ficus hispida* have poor antioxidant property. Mixture of compounds can lower antioxidant property in EA fraction of *Ficus hispida,* if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of EA fraction of *Ficus hispida.*

Further investigations can be carried out to isolate and identify the active compounds present in the plant that are responsible for pharmacological activity in the development of novel and safe drugs. Other tests can be performed to evaluate some other pharmacological activities.

CHAPTER FIVE

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