PHYTOCHEMICAL & PHARMACOLOGICAL INVESTIGATIONS ON DICHLOROMETHANE (DCM) 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, Mithila Habib Mitu, hereby declare that this dissertation, entitled "*In Vitro* Pharmacological Investigations on Dichloromethane (DCM) Fraction of *Ficus 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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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertion, entitled **"*In Vitro* Pharmacological Investigations on Dichloromethane (DCM) Fraction of *Ficus hispida* Leaf Extract"** is a bonafide research work done, under our guidance and supervision by Mithila Habib Mitu (ID: 2014-1-70-039), 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 dichloromethane (DCM) fraction of methanol extract of the leaves of *Ficus hispida*. The powdered leaves of *Ficus hispida* were extracted with methanol and then partitioned with n-hexane, dichloromethane and ethyl acetate consecutively. The DCM fraction remaining was investigated for total flavonoid content, total phenol content, brine shrimp lethality test. The fraction contained 139.167 mg AAE/gm of dried extract in total phenolic content assay and 35.3396mg 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 0.78726 μg/ml) as positive control showed that the fraction have considerable cytotoxic potency exhibiting LC_{50} value 23988329194.9045 μg/ml. The DCM fraction showed weak cytotoxic activity, low antioxidant 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 1

Introduction

1.1 MedicinalPlants

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life. (Petrovska, 2012)

Figure 1.1:Drug from medicinal plant

(Natural herbal remedies, 2017)

A medicinal plant is a plant that has same properties as customary pharmaceutical medications. Individuals have utilized these medicine plants all through history to either cure or diminish side effects from an ailment. Medicinal herbs or herbal plants are plants that are utilized for medicinal purposes. These herbs cover an extensive variety of types of plants. They can be annuals or perennials; woody or herbaceous. Find list of medicinal plants introduction, different names, herbal plants and their uses and healing plants benefits. (Sharma, 2017)

1.2History of Medicine from plants

- It is likely that humans have used plants as medicine for as long as we have existed. Archeological excavations dated as early as 60,000 years ago have found remains of medicinal plants, such as opium poppies, ephedra, and cannabis.
- Since the beginning, humans have experimented with plants to learn how they can help us heal. In essence, humans have been involved for thousands of years in a vast "clinical trial" with medicinal plants. The wisdom that resulted from this global experiment is a large part of our history of healing and healthcare.
- Presumably curative agents were discovered by trial and error. Sumerian drawings of opium from 2500 B.C. suggest that they were knowledgeable about medicinal plants. In 1770 B.C., from the Code of Hammurabi, a series of plants such as henbane (Hyoscyamusniger, Solanaceae), licorice (Glycyrrhizasp.,Fabaceae), and mints (Mentha spp., Lamiaceae) were mentioned.
- The ancient Egyptians recorded much of their knowledge of plant drugs as well. Many of the plants used by them are still used in medicine.
- The Greeks made other significant contributions to medicine. The number of effective medicinal plants came to be about 300-400 species. Hippocrates (460-377 B.C.),

Aristotle (384-322 B.C.) and Theophrastus (372-287 B.C.) essentially started the science of botany.

- The most significant contribution however, was Dioscorides (ca. 40-90 A.D.). He wrote a 5 volume work, De materiamedica that became the standard work for 1500 years. Because of later historical developments and the fact that Europe went into intellectual decline, the book was blindly followed and accepted without question until the fifteenth century.
- Finally, a contemporary of da Vinci, Paracelsus (1393-1451), broke publicly with the works of the Greeks and devised the "Doctrine of Signatures". This was soon displaced by more objective methods.
- Before the sixteenth century, most of the mainstream medical systems were based on the idea that one should work with nature and that the body's own healing capacity could be strengthened and complimented by the right herbs.
- All the old medical systems had, at their centre, a belief in a primal energy that sustained life and health. The Chinese called it "qi," while the Indians referred to it as "prana".
- Western herbalists called it the "vital force". When modern medicine took over in the nineteenth century, these concepts were dismissed as remnants of the superstition and ignorance of earlier healing practices.
- The age of western medicine had dawned and had overshadowed traditional practices in China and India.
- When the British colonized India, Ayurvedic medicine was labelled "inferior" and was subsequently squashed and replaced by western medicine.
- China was less influenced by the west medical ideas. In most European countries and the US it became illegal to practice herbal medicine without an official qualification.
- In the 19th century, such compdounds as quinine, strychnine, morphine, and ephedrine were isolated and studied. Later (mostly in the twentieth century) many of the compounds were synthesized and some became available from that source.
- Today, in western culture, most of the active ingredients are isolated, purified, and standardized, or ... ironically (in the U.S.) are sold in "Health Food" stores with little assurance that the plant materials are pure, contain the active principles, or are effective.

In Europe, particularly in Germany, companies that market these products are required to establish efficacy and to provide the materials in a form that ensures that the active materials are present in a designated dosage. In many other cultures, the crude plant drugs are still used directly.

- Most of the drugs used in western culture come from Europe and Asia, although a number of extremely important ones come from other source.

(Health24.com,2015)

1.3 Importance of Medicinal Plants:

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%.

The importance of medicinal plants is given below.

- Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.
- Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world.
- Some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.

- Herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices.
- Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively.
- As our lifestyle is now getting techno-savvy, we are moving away from nature. While we cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. (Zahid, 2016)

1.4 Active ingredients provided by Medicinal Plants

Plants produce many chemicals that are biologically active, not just in themselves, but also in other organisms. Some of these chemicals enhance their own survival. Some plants produce chemicals that act as herbicides to inhibit the growth of competing plants, such as the salicylic acid produced by willows. Other plants produce substances that deter browsing by insects and herbivores. Below are several examples of active plant ingredients that provide medicinal plant uses for humans.

Alkaloids: This group is comprised of a wide variety of plants that contain nitrogen-bearing molecules that make them very active. Many of these plants have been used to create well-known drugs used for medicinal purposes. One such example, vincristine, which was derived from the Madagascar periwinkle (*Catharanthus roseus*), is used to treat some types of cancer. Another example is atropine, which is found in deadly nightshade.

Bitters: This group is comprised of a variety of plants that are lumped together because of their very bitter taste. This bitterness causes stimulation of the salivary glands and digestive organs. As such, bitters can be used to improve appetite and strengthen the digestive system. Examples of bitters include wormwood and hops.

Cardiac Glycosides: These compounds are found in various medicinal plants (Foxglove, Lily of the Valley) and have strong direct action on the heart. Cardiac glycosides such as digitoxin, digoxin, and convallotoxin support heart strength and rates of contraction when failing. These compounds also have a diuretic effect that stimulates urine production and aids in removal of fluid from tissues and the circulatory system.

Cyanogenic Glycosides: These glycocides are based upon cyanide, a very deadly poison, but in small doses, they can serve as a muscle relaxant. The bark of wild cherry and the leaves of elderberry (*Sambucusracemosa*) contain cyanogenicglycocides, which can be used to suppress and soothe dry coughs.

Flavonoids: Flavonoids are found widely throughout the plant world and they have a wide range of medicinal uses and actions. They often act as pigments giving a yellow or white color to flowers and fruits. Some flavonoids have anti-viral and anti-inflammatory properties. Flavonoids found in many plants like lemon and buckwheat are known to strengthen capillaries and prevent leakage into tissues.

Minerals: Many plants have high levels of minerals because they can draw minerals from the soil and can convert them into a form that is more easily used by the human body. Mineral content is often the key factor in a plant's effectiveness as a medicine. One example of a plant high in minerals is horsetail. The high silica content in horsetail plants is used for arthritis because it supports the repair of connective tissue.

Phenols: Phenols are plant compounds that are thought to be produced to protect against infection and herbivory by insects. They are often anti-inflammatory and antiseptic and can have anti-viral properties. Phenols vary in structure and range from salicylic acid (similar to aspirin) to complex sugar-containing phenolic acids. Wintergreen and willow contain salicylates. Members of the mint family often contain phenols.

Polysaccharides: Polysaccharides are found in all plants and comprised of multiple units of sugar molecules linked together. For medicinal purposes, the "sticky" polysaccharides produce mucilage or gums that are commonly found in bark, roots, leaves, and seeds. These sticky

polysaccharides are able to soak up large quantities of water and form jelly like masses that can be used to treat dry or irritated tissues such as skin and mucous membranes.

Proanthocyanins: These compounds are pigments, which give fruits and flowers red, purple, or blue hues and are closely related to tannins and flavonoids. These compounds have been documented to be valuable in protection of circulation specifically in the heart, eyes, and feet. Red grapes, blackberries, and hawthorn berries all have high levels of proanthocyanins.

Saponins: This group of active compounds obtains its name from the fact that like soap, they produce lather when placed in water. There are two main forms of saponins: steroidal and triterpenoid. Steroidal saponins are very similar to the chemical structures of many of the human body's hormones including estrogen and cortisol. Examples plants containing saponins include agave, wild yam, and several members of the lily family. Several native plants are used in a process to produce synthetic hormones for humans.

Figure 1.2 : Gambel Oak (*Quercusgambellii*). Oak bark is in high tannins

Figure 1.3 :Rose hips are extremely high inVitamin C and are very delicious.

Tannins: Most plants produce tannins. Tannins serve as a deterrent to herbivory by insects and grazing animals given that that they provide a harsh unpalatable flavor. Tannins are also useful in curing leather because of their tendency to contract and astringe tissues by binding with precipitating proteins. Examples of plants high in tannins include oak bark and black catechu.

Vitamins: Many plants contain high levels of useful vitamins. Many well-known fruits and vegetables have high levels of vitamin C and beta-carotene. Lesser-known vitamin containing plants like watercress, rose hips, and sea buckthorn have high levels of vitamins B, C, and E.

Volatile oils: Volatile oilsare extracted from plants and are used to produce essential oils that play a very important role in medicinal botany. These oils are often very complex and can be comprised of 100 or more compounds. These oils have many uses. For example, tea tree oil is a strong antiseptic. Resins and gums are often linked with essential oils, however these are not volatile.(USDA, 2016))

1.5 Important Medicinal PlantsIn Bangladesh

Bangladesh has tremendous wealth of medicinal plants.Medicinal plants mainly used in the preparations of Unani and Ayurvedic medicine, also prescribed by practitioners of traditional medicine in different parts of the country and others are used as household remedies by the common people.

Table 1.1: Some Important Medicinal Plants In Bangladesh

Plant	Common name / Maturity period	Botanical Name or Family	Parts Used	Medicinal Use
	Amla (T)After 4th year	Emblicaofficinalis Fam - euphorbiaceac	Fruit	Vitamin - C, Cough , Diabetes, cold, Laxativ, hyper acidity.
	Ashok (T)10 years onward	SaracaAsoca Fam : Caesalpinanceac	Bark Flowe r	Menstrual Pain, uterine, disorder, Deiabetes.
	Aswagandha (H), One year	WithaniaSomnifera Fam: Solanaccac	Root, Leafs	Restorative Tonic, stress, nerves disorder, aphrodiasiac.
	Bael / Bilva (T)After 4-5 year	Aeglemarmelous Fam: Rutaccac	Fruit, Bark	Diarrrhoea, Dysentry, Constipation.
	BhumiAmla (H), with in one year	Phyllanthousamarus Fam : euphorbiaccac	Whole Plant	Aenimic, jaundice, Dropsy.
	Brahmi (H) Indian penny worth/one year	Bacopa,Monnieri Fam: Scrophulariaccac	Whole plant	Nervous, Memory enhancer,mental disorder.

	Chiraita (high altituted) with in one year (H)	SwertiaChiraita Fam : Gentianaccac	Whole Plant	Skin Desease, Burning, censation, fever.
	Gudmar / madhunasini, after Four year (C)	GymnemaSylvestre Fam: Asclepiadaccac	Leaves	Diabetes, hydrocil, Asthama.
	Guggul (T)after 8 years	CommiphoraWightii Fam: burseraccac	Gum rasine	Rheumatised, arthritis, paralysis, laxative.
	Guluchi / Giloe (C)With in one year	TinosporaCordifoliaFam	Stem	Gout, Pile, general debility, fever, Jaundice.
	Calihari / panchanguliaGlori Lily Five years	Gloriosasuperba Fam: Liliaccac	Seed, tuber	Skin Desease, Labour pain, Abortion, General debility.
	Kalmegh/ Bhuineem (H) with in one year	AndrographisPaniculataFam : scanthaccac	Whole Plant	Fever, weekness, release of gas.
	Long peeper / Pippali (C) after two to three years	Peeper longum Fam : Piperaccac	Fruit, Root	Appetizer, enlarged spleen, Bronchities, Col

(Bokhtearuddin S, 2016)

1.6 Dumur, Hairy fig,*Ficushispida*

1.6.1 Bengali/vernacular name:

Kakdumur, Khoksha-dumur, Dumur, Dhungri, Thoska.

1.6.2 Tribal name:

Tammanggaas (Tanchangya), Dhumurgula (Chakma), Fahshaiba (Marma), Thainjang (Tripura), Luhuk (Murong)

Figure 1.4 :Dumur Tree

(Easy Ayurveda, 2014)

1.6.3 Taxonomical classification

Domain: Eukaryote

Kingdom: Plantae

Division: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: *hispida*

(Ali and Chowdhury, 2011)

1.6.4 Morphology

Fig plant is an ornamental, flowering and also a fruit plant, which belongs from family of "Moraceae". Scientific name of this plant is "*Ficus hispida*" and it also known as common fig. It is an important crop and used for commercially reason. It produces flower, which are unisexual and fruits, which are one seeded. Fig has a big store of calcium, vitamin, magnesium and fibers etc.

Full sunlight is good for fig plants growth and it can helps to ripe fruit in one day. It grows in warmth weather and it needs well-drained soil, which is mix with quite sand, medium loam and heavy clay. It needs moisture soil and for keeping moisture it needs regular watering for its well growth. Regular fertilizing is must be need for this plant growth and once or twice in a week is preferable for this plant. For nitrogen, it needs nitrogen fertilizer for its well growth.

Flowers remain inside fruit and cannot be seen from outside. Fruits called figs grow in cluster on branches. The ripe fruit is yellow. Ovulate green leaves are hairy and rough. Leaf is 12-18 cm long and pedicel is 2-5 cm long.

Figure1.5 : Leaves of *Ficus hispida*

Figure1.6 : propagation is by seeds

Ripe fruits are eaten by birds and seeds are spread with their droppings.s are spread with their droppings.

(Flora of Bangladesh.com, 2015)

1.6.5Distribution

Ficus hispida Linn (FH) belongs to the family Moraceae is a widely distributed ant most commonly found in the interior and coastal regions distributed throughout India, Srilanka, Myanmar,Southern region of the Republic china, New guinea, Australia and Andaman Islands in damp localities, it grows in secondary forests, open lands and river banks, up to 1200 m in altitude.

1.6.6 Cultivation Details

Fig trees have a unique form of fertilization, each species relying on a single, highly specialized species of wasp that is itself totalydependant upon that fig species in order to breed. The trees produce three types of flower; male, a long-styled female and a short-styled female flower, often called the gall flower. All three types of flower are contained within the structure we usually think of as the fruit.

The female fig wasp enters a fig and lays its eggs on the short styled female flowers while pollinating the long styled female flowers. Wingless male fig wasps emerge first, inseminate the emerging females and then bore exit tunnels out of the fig for the winged females. Females emerge, collect pollen from the male flowers and fly off in search of figs whose female flowers are receptive. In order to support a population of its pollinator, individuals of a Ficus spp. must flower asynchronously. A population must exceed a critical minimum size to ensure that at any time of the year at least some plants have overlap of emmission and reception of fig wasps. Without this temporal overlap the short-lived pollinator wasps will go locally extinct.

(Topical plants, 2014)

1.6.7 Chemical constituents:

- Bark contains tannins and saponin glycosides.
- Leaves contain bergapten, psoralen, β-amyrin and β-sitosterol.
- Fruits contain protein, ascorbic acid, carbohydrates and minerals.
- The plant also contains 10-keto-tetracosylarachidate. Hydrocarbons present on the leaf surface have also been studied.

(Ghani, 2003)

A new norisoprenoid, ficustriol, and the known phenanthroindolizidine alkaloid *O*-methyltylophorinidine, were isolated from a $CHCl_3$ extract of the leaves and twigs of *Ficus hispida*.

1.6.8 Medicinal uses of *Ficushispida*:

- 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 Ficushispida 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 Ficushispida is given in a dose of 50 ml to treat jaundice, piles and distention of abdomen.
- The fruit juice of ripened Ficushispida 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 Ficushispida is given with jiggery to induce purgation as part of treatment.
- To treat diarrhea, decoction of bark of Ficushispida 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 (Mucunaprurita) powder is used as nasal snuf

(Easy Ayurveda, 2014)

Chapter 2

Literature Review

2.1 Pharmacognostic and ethnomedicinal properties on *Ficus hispida*

Ficus hispida (FH) Linn. is a moderate sized tree found throughout the year and is grown wild or cultivated for its edible fruits and folklore value. Traditionally, different parts of the plant have been used in the treatment of ulcers, psoriasis, anemia, piles jaundice, vitiligo, hemorrhage, diabetes, convulsion, hepatitis, dysentery, biliousness, and as lactagogue and purgative. FH contains wide varieties of bioactives from different phytochemical groups like alkaloids, carbohydrates, proteins and amino acids, sterols, phenols, flavonoids, gums and mucilage, glycosides, saponins, and terpenes. Various scientific works have been published to establish the scientific basis of traditional medicinal values attributed to FH. Furthermore, newer pharmacological activities like antineoplastic, cardioprotective, neuroprotective and anti-inflammatory effects were also reported recently. Till now, no work has been published to

elaborate the pharmacognostic features of FH Linn. The present review is, therefore, an effort to give a detailed account on its pharmacognosy and phytochemistry, and an extensive survey on its pharmacological activities. Moreover, we are trying to establish the mechanism of action behind its earlier reported pharmacology. The review also looks at the future formulation based delivery approaches of its lipophilic bioactives, which is done to enhance its dissolution so as to increase its bioavailability, and thus the associated pharmacological action.

2.2 Antinociceptive Activity Studies with Methanol Extracts of Ficus Hispida Leaves and Fruits in Swiss Albino Mice

The methanol extracts of Ficus hispidaL.f. leaves and fruits were evaluated for antinociceptive activity in acetic acid-induced gastric pain writhings in Swiss albino mice. The methanol extract of leaves demonstrated dose-dependent and significant antinociceptive activity when administered at doses of 50, 100, 200, and 400 mg per kg body weight. At these doses, the percent inhibitions of writhings versus control animals (i.e. animals without any extract administration) were, respectively, 65.71, 67.14, 70.19, and 85.05. In comparison, the standard antinociceptive drug, aspirin, when administered at a dose of 400 mg per kg body weight reduced writhings by 88.09%. The methanol extract of fruits also exhibited significant dose-dependent writhings in mice, when administered at doses from 50 to 400 mg per kg body weight. However, in the case of fruit extract, the percent inhibition of writhings was lower than corresponding doses of leaf extract. Writhings were reduced by 49.24, 61.24, 62.67, and 65.19%, respectively, when mice were administered fruit extract at doses of 50, 100, 200, and 400 mg per kg body weight. The results suggest that both leaf and fruit extract of Ficus hispida contain strong antinociceptive components, which warrant further studies to be made on these extracts towards discovery of efficacious pain relieving agents.

2.3 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.

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 μg/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.

2.5 Antihyperglycemic Activities of Leaves of Three Edible Fruit Plants (*Averrhoacarambola, Ficus hispida and Syzygium samarangense*) of Bangladesh

Averrhoacarambola L.(Oxalidaceae), *Ficushispida* L.f.(Moraceae),and *Syzygiumsamarangense* (Blume) Merr. & L.M. Perry (Myrtaceae) are three common plants in Bangladesh, the fruits of

which are edible. The leaves and fruits of *A. carambola* and *F. hispida* are used by folk medicinal practitioners for treatment of diabetes, while the leaves of *S. samarangense* are used for treatment of cold, itches, and waist pain. Since scientific studies are absent on the antihyperglycemic effects of the leaves of the three plants, it was the objective of the present study to evaluate the antihyperglycemic potential of methanolic extract of leaves of the plants in oral glucose tolerance tests carried out with glucose-loaded mice. The extracts at different doses were administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (p.o.) using glucose oxidase method. Significant oral hypoglycemic activity was found with the extracts of leaves of all three plants tested. The fall in serum glucose levels were dose-dependent for every individual plant, being highest at the highest dose tested of 400 mg extract per kg body weight. At this dose, the extracts of A. carambola, *F. hispida*, and *S. samarangense* caused, respectively, 34.1, 22.7, and 59.3% reductions in serum glucose levels when compared to control animals. The standard antihyperglycemic drug, glibenclamide, caused a 57.3% reduction in serum glucose levels versus control. Among the three plants evaluated, the methanolic extract of leaves of *S. samarangense* proved to be the most potent in demonstrating antihyperglycemic effects. The result validates the folk medicinal uses of *A. carambola* and *F. hispida* in the treatment of diabetes, and indicates that the leaves of S. samarangense can also possibly be used for amelioration of diabetes-induced hyperglycemia.

2.6 Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats

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.

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 the standard 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.

FH has significant hypoglycemic activity. Increased glycogenesis and enhanced peripheral uptake of glucose are the probable mechanisms involved in its hypoglycemic activity.

2.7 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.

2.8 Hepatic Perturbations Provoked by Azathioprine: A Paradigm To Rationalize The Cytoprotective Potential of *Ficus Hispida* Linn

The present study was embarked upon in an endeavor to ascertain whether *Ficus hispida*leaf extract (FHLE) modulates azathioprine-induced hepatic damage. Azathioprine treated rats displayed a plethora of pathological events, which include loss of hepatocellular membrane

integrity, mitochondrial dysfunction, and nuclear damage; whilst FHLE pretreated rats significantly precluded these abnormalities. These data were in harmony with the transmission electron microscopic studies. Observations from this investigation directed us to propose the plausible mechanisms through which FHLE thwarts the repercussions of azathioprine-induced hepatocellular necrosis: upholding of thiol homeostasis, curtailing the membrane effects, and perpetuation of adenine nucleotide status. These data offer credence to the notion that FHLE might be a beneficial intervention in the prevention of hepatotoxicity in azathioprine therapy.

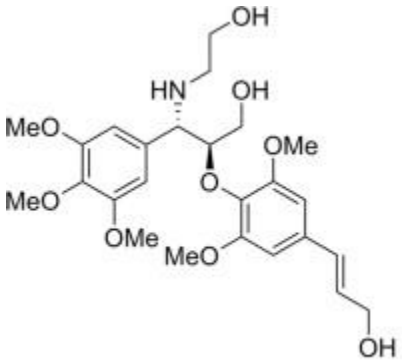
2.9ACETYLCHOLINE ENHANCING ACTIVITY OF METHANOL LEAF EXTRACT OF FICUS HISPIDA LINN IN RAT HIPPOCAMPUS

The aim of the study is to evaluate the acetylcholine enhancing activity of methanol leaf extract of Ficus hispida Linn (MEFH). Treatment with 200 and 400 mg/kg of MEFH, for 30 days to postnatal (7 days old) and young adult rats, significantly increased acetylcholine (ACh) content in their hippocampus as compared to age matched controls. It was observed that increase in ACh content in hippocampus may be the neurochemical basis for their improved learning and memory. Conclusively, MEFH was found to possess acetylcholine enhancing properties and could act as a potent therapeutic agent against age related memory disorders.

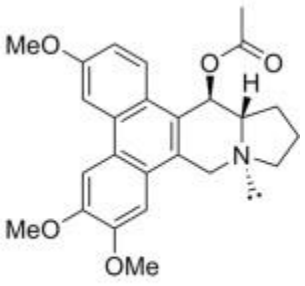
2.10 Hispidacine, an unusual 8,4′-oxyneolignan-alkaloid with vasorelaxant activity, and hispiloscine, an antiproliferativephenanthroindolizidine alkaloid, from *Ficus hispida* Linn.

Hispidacine, an 8,4′-oxyneolignan featuring incorporation of an unusual 2-hydroxyethylamine moiety at C-7, and hispiloscine, a phenanthroindolizidine alkaloid, were isolated from the stem-bark and leaves of the Malaysian *Ficus hispida* Linn. Their structures were established by spectroscopic analysis. Hispidacine induced a moderate vasorelaxant activity in rat isolated aorta, while hispiloscine showed appreciable antiproliferative activities against MDA-MB-231, MCF-7, A549, HCT-116 and MRC-5 cell lines.

Phytochemical investigation of a Malaysian sample of *Ficus hispida* Linn. led to isolation of an unusual 8,4′-oxyneolignan-alkaloid, hispidacine, and two phenanthroindolizidine alkaloids, hispiloscine and (+)-deoxypergularinine. Hispidacine induced vasorelaxant activity in rat aorta, while hispiloscine showed antiproliferative activities against four human cancer cell lines.



Hispidacine



Hispiloscine

2.11Evaluation of Nephroprotective Activity of Fruits of *Ficus hispida* on Cisplatin-Induced Nephrotoxicity

Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet. In spite of overwhelming influence of modern science and tremendous advances made in the production of synthetic drugs, traditional medicaments designed as herbal drugs in different places in the literature have retained their place in the therapy. In this context the folk medicine *Ficus hispida* was studied for its nephroprotective activity. The nephroprotective effect of fruits of *Ficus hispida* (Moraceae) were investigated by acute toxicity studies, estimation of biochemical parameters and *in vitro* antioxidant studies. In our investigation methanolic extract showed significant nephroprotective activity than nephrocuration on cispaltin induced nephrotoxicity.

2.12 Studies on anti-diarrhoeal activity of *Ficus hispida*. Leaf extract in rats

Methanol extract of *Ficus hispida* L. showed significant inhibitory activity against castor oil-induced diarrhoea and PGE_2-induced enteropooling in rats. It also showed a significant reduction

in gastro-intestinal motility on charcoal meal test in rats. The results obtained establish the *F. hispida* leaf extract as an anti-diarrhoeal agent.

2.13 Sedative and anticonvulsant activities of the methanol leaf extract of Ficus hispida Linn.

The central nervous system (CNS) depressant and anticonvulsant activities of the methanol leaf extract of Ficus hispida Linn (FH) were investigated on various animal models including pentobarbitone sleeping time and hole-board exploratory behaviour for sedation tests, and strychnine, picrotoxin, and pentylenetetrazole-induced convulsions in mice. FH (200 and 400mg/kg, p.o.), like chlorpromazine HCl (1mg/kg, i.m.), produced a dose-dependent prolongation of pentobarbitone sleeping time and suppression of exploratory behaviour. FH (200 and 400mg/kg) produced dose-dependent and signi?cant ($P < 0.05$) increases in onset to clonic and tonic convulsions, and at 400mg/kg, showed complete protection against seizures induced by strychnine and picrotoxin but not with pentylenetetrazole. Acute oral toxicity test, up to 14 days, did not produce any visible signs of toxicity. These results suggest that potencially antiepileptic compounds are present in leaf extract of FH that deserve the study of their identity and mechanism of action.

Chapter 3

Methods and Materials

3.1 Collection & Preparation of Plant Material:

Plant sample (Leaves) of*Ficushispida* was collected from Dohar, Dhaka in March 2016. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Extraction of the plant material:

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

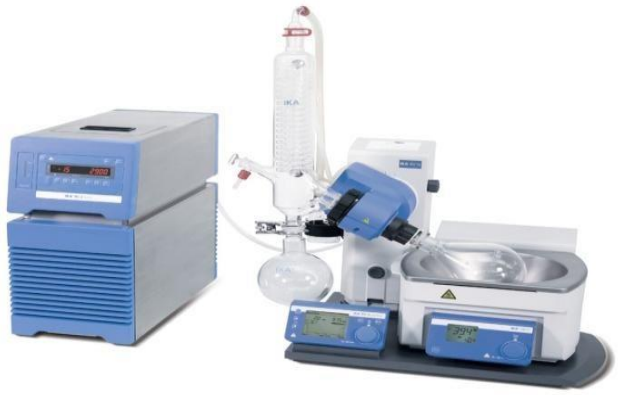


Figure 3.1:Drying of extract using rotary evaporator

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

3.3 Preparation of Mother Solution:

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.

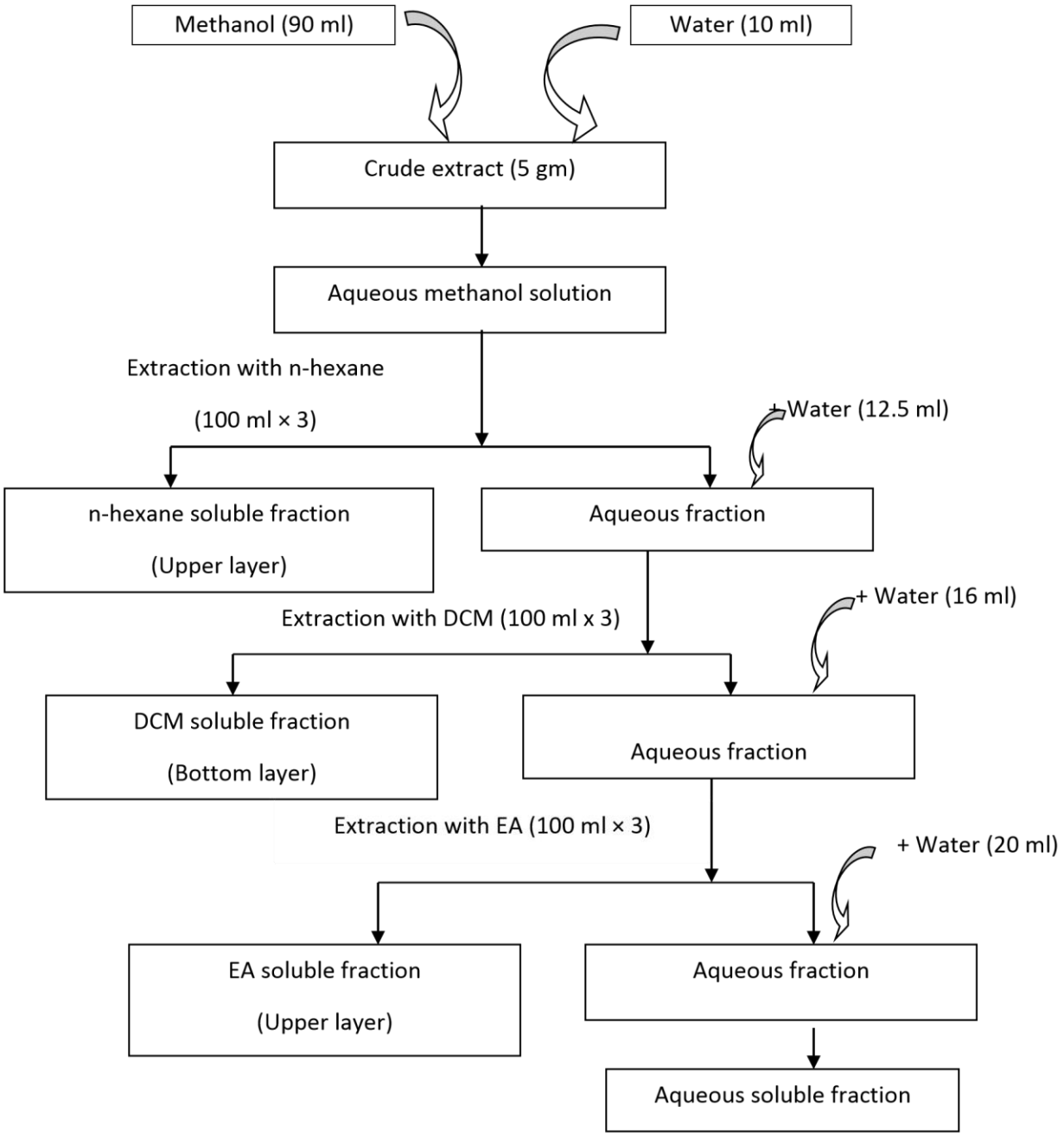


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Ficus hispida*leaves

3.4.1 Partition with n-Hexane:

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 n-hexane fraction was then air dried for solid residue.

3.4.2 Partition with Dichloromethane:

To the mother solution left after partitioning with n-hexane, 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 was then air dried for solid residue.

3.4.3 Partition with Ethyl acetate :

To the mother solution that left after washing with n-hexane, 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 Fractionwith Aqueous :

After partitioning the mother solution with n-hexane 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.

3.4.5 Collection of DCM Fraction:

After partitioning the mother solution with the four different solvents the DCM fraction was collected and air dried. This DCM was further investigated for different pharmacological properties (antioxidant, cytotoxic and antimicrobial).

3.5 Antioxidant Activity:

3.5.1 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 antioxidants 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 antioxidative effects have rarely been carried out. The purpose of this study was to evaluate extractives of Opuntiaelatior as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity. 50 Cytotoxic and Antioxidant activity in aqueous fraction of Opuntiaelatior extract

3.5.1.1 Principle:

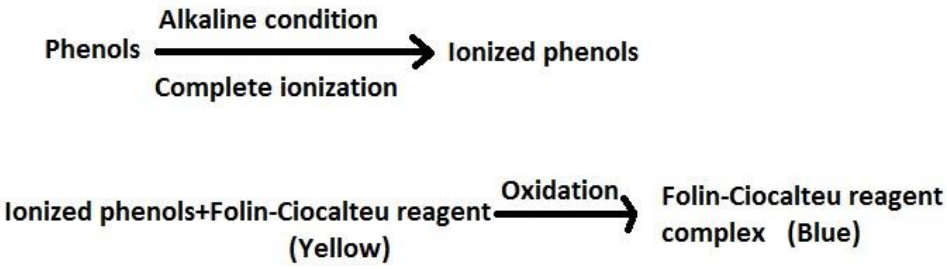
The content of total phenolic compounds in plant methanolic extracts was determined by Folin–

Ciocalteu Reagent (FCR). The FCR actually measures a sample’s reducing capacity. In the alkaline condition phenols ionize completely.

Table 3.1: **Composition of 100 mg Folin-Ciocalteu Reagent:**

Water	57.5 ml
Lithium Sulfate	15.0 mg
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

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 poly phosphotunstates - molybdates. Sequences of reversible oneor two-electron reduction reactions lead to blue species, possibly (PMoW11O40) 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).



3.5.1.2 Apparatus & Reagents:

Table 3.2: Apparatus and reagents used for total phenolic content:

Folin-Ciocalteu reagent (10 fold diluted)	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Na2CO3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200 µl)
Distilled water	Cuvette

3.5.1.3 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 Na2CO3 (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 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.

3.5.2 Total Flavonoid Content:

3.5.2.1 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 C et al., 2002). Flavonoid

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

3.5.2.2 Apparatus&Reagents :

Table 3.3: Apparatus and reagents used for total flavonoid content:

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

3.5.2.3 Procedure:

Preparation of 10% Aluminium Chloride (Alcl3) Solution: 10 mg of AlCl3 was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of 4% NaOH Solution: 4 mg 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: 5 mg of NaNO2 was taken into a 100 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. The experimental concentrations were prepared from this stock solution.

Table 3.4: Preparation of standard solution:

Concentration (µg/ml)	**Solution taken from stock solution (ml)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
0	0.0	5	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 6 minutes. 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. Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank.

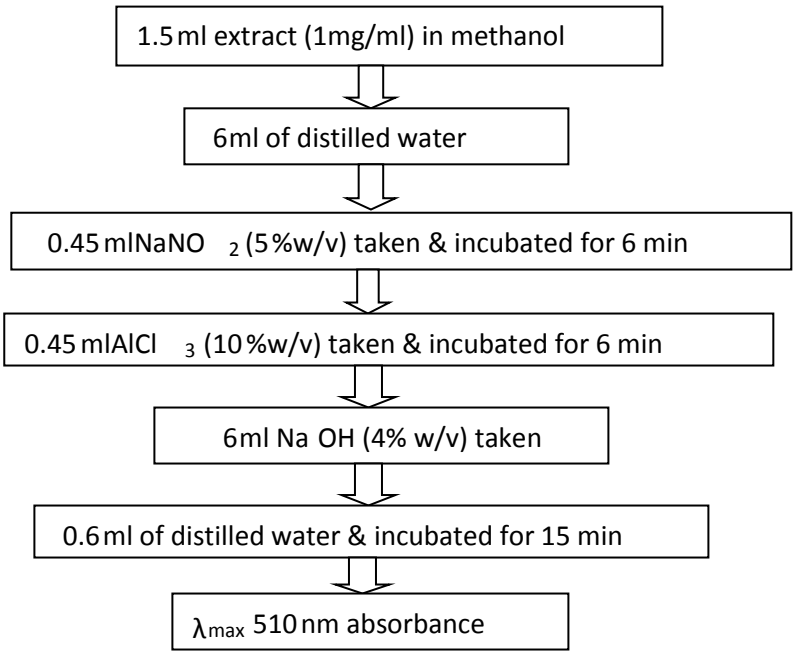


Figure 3.3:Schematic diagram of preparation of extract solution

Preparation of blank solution:

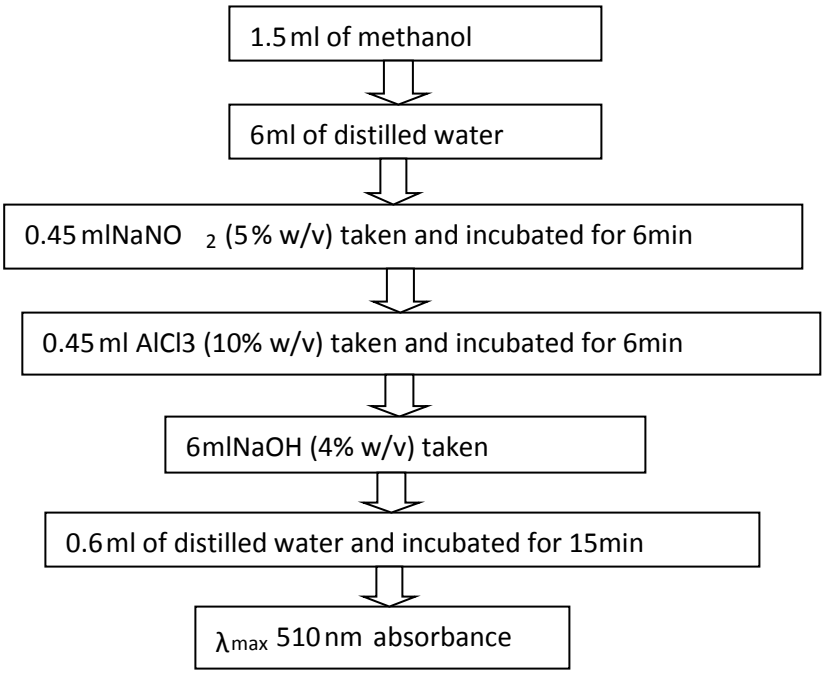


Figure 3.4: Schematic diagram of preparation of blank solution

3.6 *Brine Shrimp* Lethality Bioassay:

3.6.1 Principle:

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism, (Brine shrimp napulii- Artemiasalina) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae (Olowa and Nuneza, 2013).

3.6.2 Apparatus & Reagents:

Table 3.5: Apparatus and reagents for Brine shrimp lethality bioassay:

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

3.6.3 Procedure:

3.6.3.1 Preparation of Sea Water:

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

3.6.3.2 Hatching of Brine Shrimp:

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

freshly hatched free -swimmingnauplii were used for the bioassay.

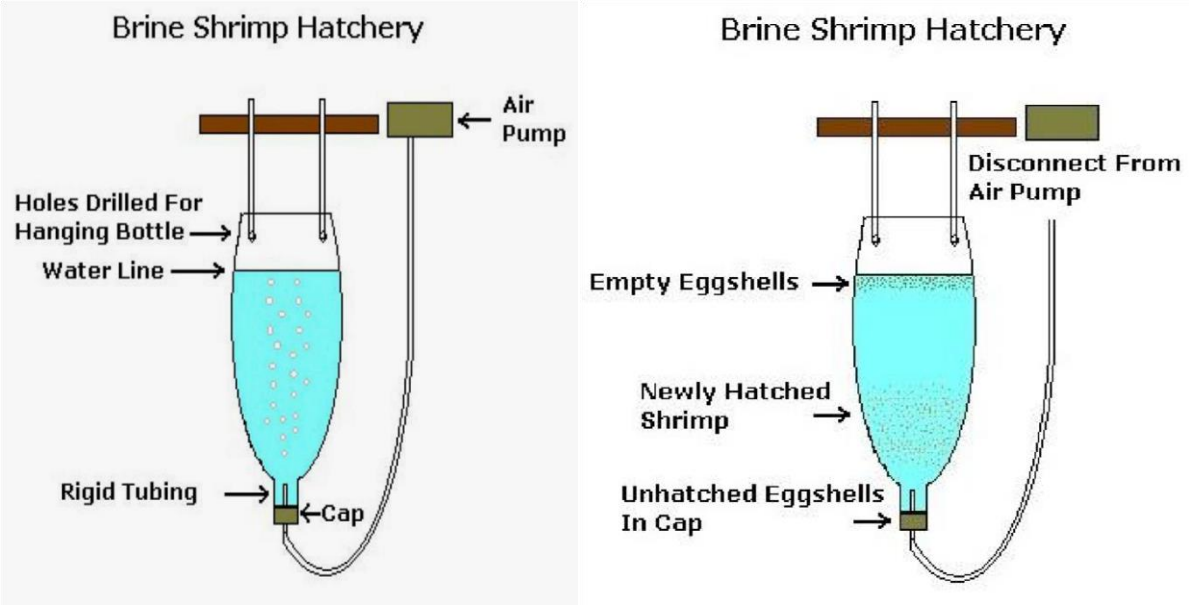


Figure 3.5: Brine shrimp Hatchery

3.6.3.3 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.

3.6.3.4 Experimental Plant:Preparation of The Test Samples of *Ficus hispida*

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

50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml for 10 dilutions.

3.6.3.5 Preparation of the Positive Control Group:

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 20µg/ml. From that stock solution serial dilutions are made using DMSO to get 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml. Then ten living brine shrimp nauplii in 5ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.6.3.6 Preparation of the Negative Control Group:

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

3.6.3.7 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.

Chapter 4

Result And Discussion

4.1 Result of Brine Shrimp Lethality Bio-Assay

The DCM fraction 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 DCM 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 naupliidead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	6	4	40	0.78726
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	1	9	90	

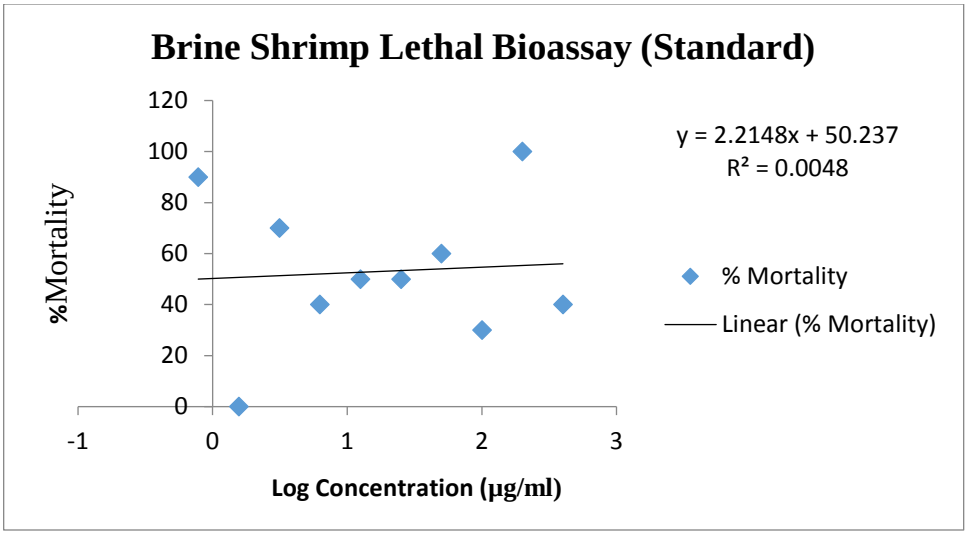


Figure 4.1: % Mortality and predicted regression line of Tamoxifen (standard)

4.1.2 Preparation of DCM Fraction Curve

Table 4.2: Results of the bioassay of DCM 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	
2	200	2.301	2	8	80	
3	100	2.000	1	9	90	
4	50	1.699	0	10	100	
5	25	1.398	2	8	80	

6	12.5	1.097	0	10	100	23988329
7	6.25	0.796	1	9	90	194.9045
8	3.125	0.495	0	10	100	
9	1.5625	0.194	0	10	100	
10	.078125	-0.107	0	10	100	

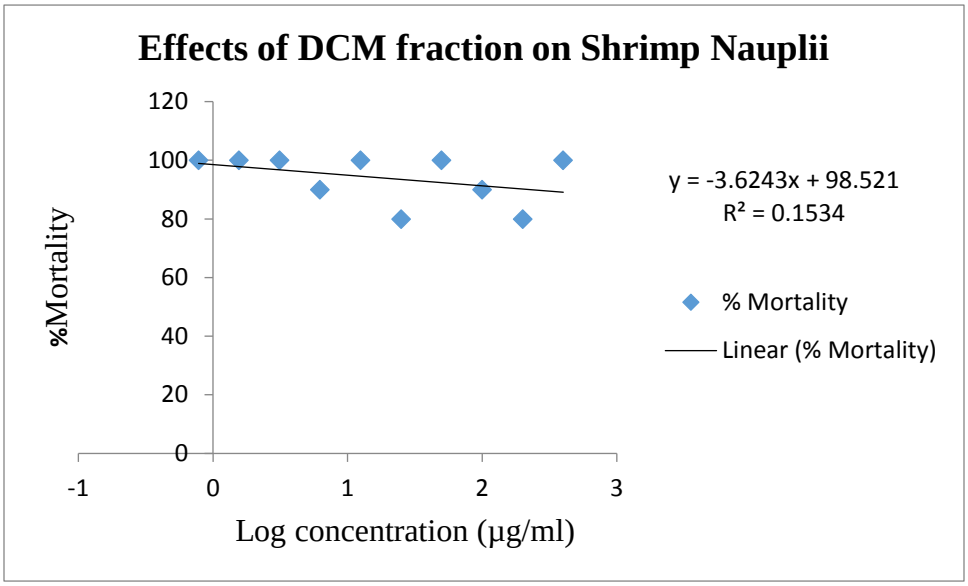


Figure 4.2: % Mortality and predicted regression line of DCM extract

4.1.3 Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was not found to be directly proportional to the concentration ranging from the lowest concentration to the highest concentration in both standard and DCM fraction samples. Mortality is not increased gradually with an increase in concentration of the test samples. Maximum mortalities took place at the highest concentration of 400µg/ml, whereas the least mortalities at lowest concentration 200µg/ml as shown in Table 4.1 and Table 4.2.

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

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml, 24hr)
Standard (Tamoxifen)	y = 2.214x+50.23	0.004	0.78726
Extract (DCM fraction)	y = -3.624x+ 98.52	0.153	23988329194.9045

In this investigation, standard and DCM fraction exhibited cytotoxic activities with the LC_{50} values 0.78726µg/ml and 23988329194.9045µg/ml respectively as shown in Table 4.3. For DCM fraction, LC_{50} value is more than the standard which indicates that the extract has less potent activity than standard against brine shrimp nauplii.

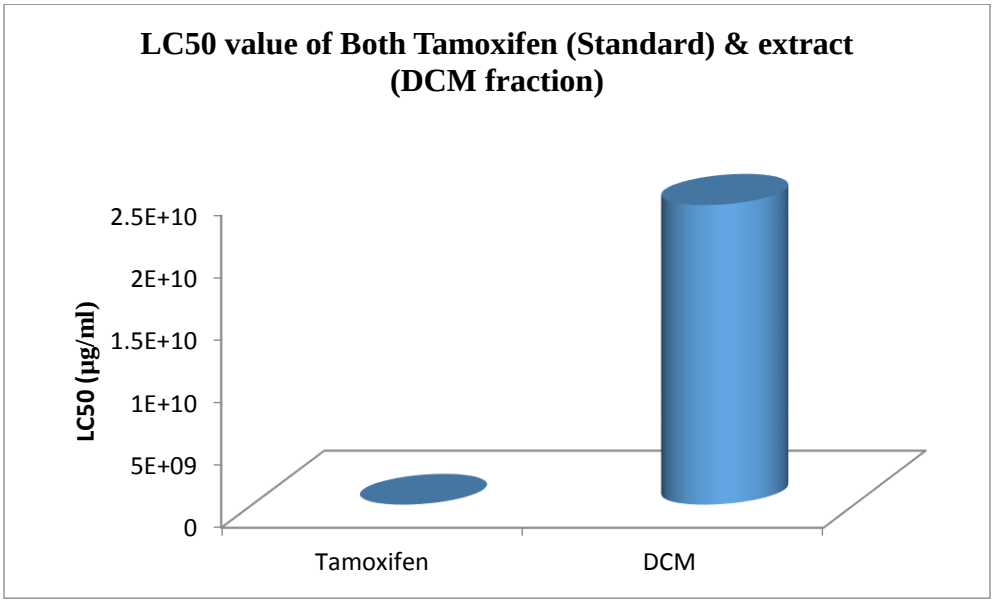


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for aqueous fraction the lethal concentration required to kill 50% of the sample population is higher than the standard. So the extract is less 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 DCM 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

The DCM extract of *Ficus hispida* and the DCM fractions of the methanol extract of *F. hispida* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard

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

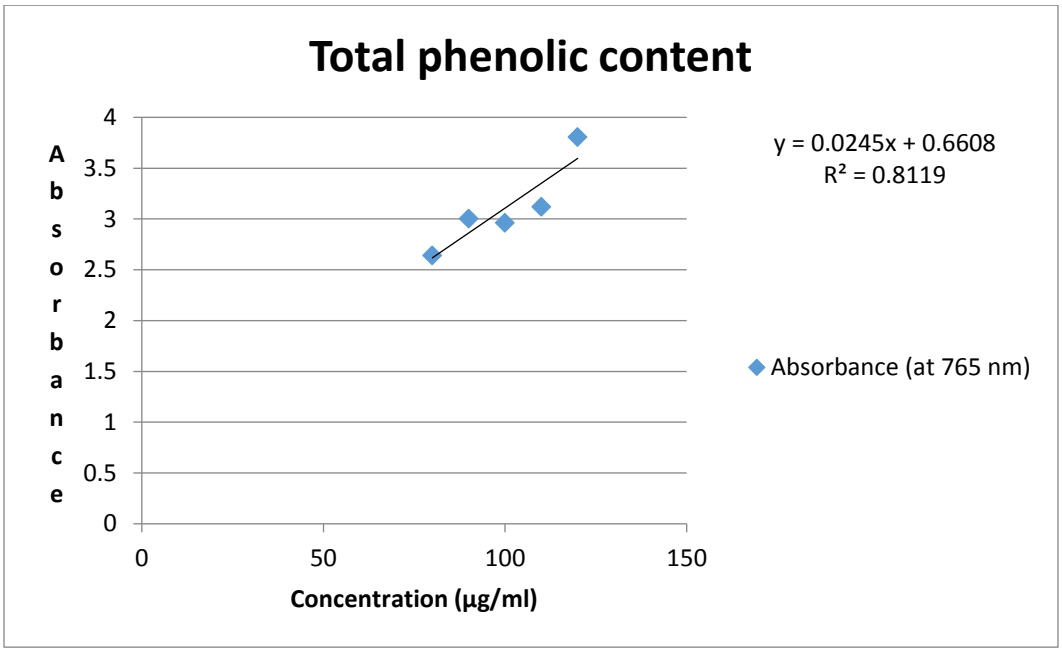


Figure 4.4: Graphical representation of Phenolic content of ascorbic acid

4.2.1.2 Total Phenolic content present in DCM extract of *F.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 DCM fraction of *F.hispida*

Concentration (mg/ml)	**Absorbance**	**mg AAE/g**
2	4	139.167

4.2.1.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the DCM fraction is more 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 DCM fraction of *Ficus hispida.*

4.2.2 Result of Total Flavonoid Content

The DCM fractions of *Ficus 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.

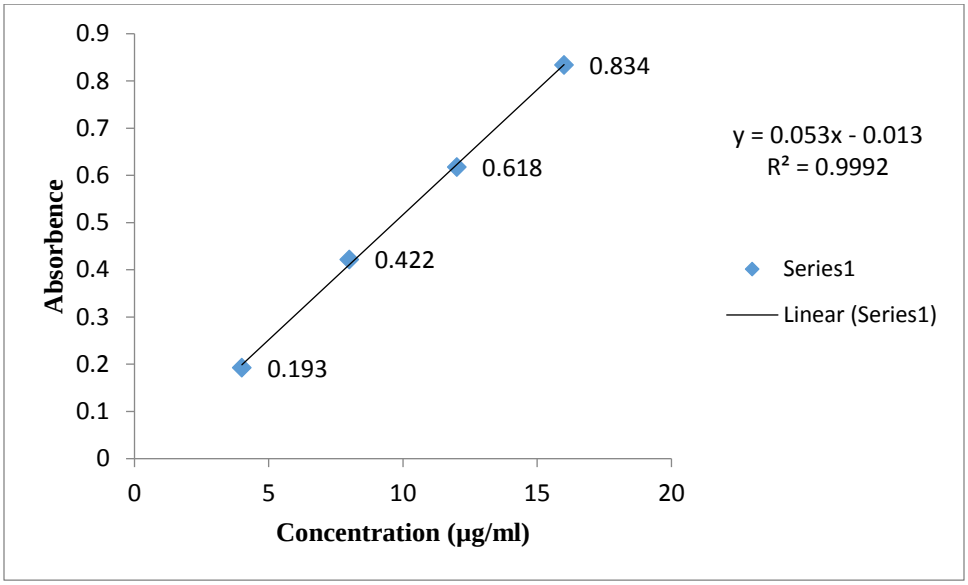


Figure 4.5: Graphical representation of assay of flavonoid content of quercetin

4.2.2.2 Total Flavonoid Content Present in Aqueous Extract

Based on the absorbance value of extract solution and using the regression line equation of the standard curve, the total flavonoid present in the extract is calculated and is given in Table 4.7

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

Sample	**Concentration (mg/ml)**	**Absorbance**	**Total flavonoid content (mg of quercetin/g of dried extract)**
DCM fraction of *F. hispida*	1	1.86	35.3396

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 DCM fraction of *Ficus hispida* (leaves), 35.3396 mg of quercetin/gm of

dried extract of flavonoid content was found. So it can be said that, the extract contains very low antioxidative compounds.

CHAPTER 5

Conclusion

5.1 Conclusion

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

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

Antioxidant property in aqueous extract of *F. hispida* was determined by Phenolic content assay and Flavonoid content. Phenolic content was 139.167 mg/gm and Flavonoid content 35.3396 was mg/gm in DCM extract of *F. hispida.* So DCM extract of *Ficus hispida* have poor antioxidant property. Mixture of compounds can lower antioxidant property in DCM fraction of *F.hispida,* if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of DCM fraction of *F. 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-6

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