Antioxidant and Cytotoxic activity of crude extract of *Opuntia elatior* Mill

A Dissertation submitted for the partial fulfillment of the course of Pharmaceutical Research (PHRM-404) of the Department of Pharmacy, East West University, for the degree of Bachelor of Pharmacy.

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Declaration by the Research candidate

I,Shawon Majumder, hereby declare that this dissertation, entitled **"**Antioxidant and Cytotoxic activity of crude extract of *Opuntia elatior* mill" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (B.PHRM) is a complete record of original research work carried out by me during the period 2014-2015 under the supervision and guidance of Abdullah-Al-Faysal, Lecturer, Department of Pharmacy, East West University, Dhaka. And it has not formed the basis for the award of any other Degree/Diploma/Fellowship or other similar title to any candidate of any university.

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ABSTRACT

The purpose of the study was to evaluate the antioxidant and cytotoxic activity of crude extract of *Opuntia elatior* mill (Family: Cactaceae).

The powdered of *Opuntia elatior* mill were extracted with methanol and then partitioned with pet-ether, DCM, ethyl acetate and crude fraction was taken for experiment.

The crude extract was used to evaluate cytotoxic and antioxidant activities. The cytotoxic activity was measured by brine shrimp lethality bioassay.

.Screening for cytotoxic properties using brine shrimp lethality bioassay with tamoxifen (LC_{50} value of 25µg/ml) as positive control showed that the fraction have considerable cytotoxic potency exhibiting LC50 value 6.25µg/ml.

The crude extract contained 23.895 mg AAE/g of total phenolic content, 111.1 mg AAE/g of total reducing power content and 58 mg AAE/g total flavonoid content.

The results of study clearly indicate the presence of cytotoxic and antioxidant properties of crude extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: *Opuntia elatior* mill, Brine shrimp lethality bio-assay, phenolic content, flavonoid content, reducing power assay.

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INTRODUCTION

1.1 Introduction:

Throughout the ages humans have relied on nature for their basic needs for the production of foodstuffs, shelter, clothing, means of transportation, fertilizers, flavors and not least, medicines. Nature has been a source of several medicines for treating various types of diseases in humans and animals for many years (Kapoor, 2001).

Plants are the important sources of a diverse range of chemical compounds. Some of these compounds possessing a wide range of pharmacological activities are either impossible or too difficult to synthesize in the laboratory. A phytochemist uncovering these resources is producing useful materials for screening programs for drug discovery. Emergence of newer diseases also leading the scientists to go back to nature for newer effective molecules (But and Chang, 2001)

Plants have formed the basis for traditional medicine systems which have been used for thousands of years in countries such as China and India. The use of plants in the traditional medicine of many other cultures has been extensively documented. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care.[3] Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. In a study it has been shown that at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs that are in use in one or more countries. Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine (Arvigo and Balick, 1993).

Examples of traditional medicine providing leads to bioactive natural products abound. Suffice it to point to some recent confirmations of the wealth of this resource. Artemisine (1, Figure 1) is the antimalerial sesquiterpene from a Chinese medicinal herb *Artemisia annua* (wormwood) used in herbal remedies since ancient times. Forskolin is the antihypertensive agent from *Coleus forskohlii* Briq. (Labiatae), a plant whose use was described in ancient Hindu Ayurvadic texts (Bhat, Bajwa, 1977).

Paclitaxel is the most recent example of an important natural product that has made an enormous impact on medicine. It interacts with tubulin during the mitotic phase of the cell cycle, and thus

prevents the disassembly of the microtubules and their by interrupts the cell division.The original target diseases for the compound were ovarian and breast cancers, but now it is used to treat a number of other human tissue proliferating diseases as well (Strobel, Daisy, 2004).

A case of serendipity is the discovery of the so called vinca alkaloids, vincristine (4) and vinblastin (5), in *Catharanthus roseus*. A random screening program (conducted at Eli Lilly and Company) of plants with antineoplastic activity found these anticancer agents in the 40^{th} of 200 plants examined. Ethnomedicinal information attributed an anorexigenic effect (i.e. causing anorexia) to an infusion from plant (Tyler, 1986).

In recent years, there is growing interest in the therapeutic use of natural products, especially those derived from plants, due to several reasons including 1.Conventional medicines have more side effects and ineffective in therapeutical use, 2. Abusive or incorrect use of synthetic drugs may cause many problems and side effects, 3. A huge number of populations in the world not depend on conventional pharmacological treatment, and 4. Folk medicine and ecological awareness suggest that "natural" products are harmless (Sarker *et al*, 2006).

Within the next quarter century, the achievements of science and technology will be so great that, when brought to bear upon the mysteries of nature that have long puzzled us those mysteries will yield their secrets with amazing rapidity. It will be a fascinating and eventful period. We will not know only the causes of disease but the cures for most. Significant new drugs of plant origin and new methods of producing them will continue to be important parts of that service and thus Plants are considered as are of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidate (Sarker *et al*, 2005).

1.2 Research of Traditional drug in Bangladesh

In rural areas medicinal plants have been being used as remedy for disease for a long time. They not only cure the disease but also provide an important role in the economy. Medicinal plants are cheap and easy to get to those people who knew it very well. Bioactive compounds are deposited in medicinal plants; it can serve as important raw materials for pharmaceutical manufacturing. They comprise a precious asset of a country and donate to its health care system (Husain, 1992).

Well-judged and scientific investigation of this wealth can significantly contribute to the public health. More than 500 of medicinal plants have so far been established as growing in Bangladesh. Almost all of these indigenous medicinal plants are extensively used in the preparation of unani, ayurvedic and homeopathic medicines in Bangladesh.

A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% of people suffering illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mullahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in crude and substandard form, by our people. In fact, a survey report concluded that 39% of rural community members have knowledge about medicinal plants and 13% treat simple ailments with herbs (Ghani, A, 2003).

Traditional medicines are still manufactured in our country by following the age-old unscientific, traditional methods. Hundreds of indigenous medicinal plants are employed in different Ayurvedic and Unani commercial preparations without proper standardization, quality control, evaluation and determination of the chemical nature, pharmacological and toxicological studies of the active components which are essential to utilize their therapeutic potential fully. Toxicity of the plants or plant extracts is coming to light with the advancement of science. Since Bangladesh is a country of low economic growth, a proper health care system can be established by supplying low cost medicines to its population. This may be possible only by developing standard drugs from our natural resources of medicinal plants. In order to achieve this goal, research and development of traditional medicines should be given the due priority. Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to manufacture drugs. Each year a great deal of money is spent on this purpose (Ghani, A, 2003).

1.3 Phytochemistry

Phytochemistry is the name given to the study of the chemistry of plants. It is in the strict sense of the word the study of phytochemicals. These are chemicals derived from plants. Like animals, plants produce a wide variety of chemical compounds, called metabolites, as part of their normal life processes. These compounds perform different functions. For example, some enable plants to

store energy in the form of sugar, whilst others are protective against disease or predators. The Phytochemistry Unit functions as a laboratory for herbal/plant collection and processing, sample preparation and chromatographic analysis of extracts, fractions and compounds isolated from herbs and medicinal plants (Phytochemistry,2012).

1.4 Selection, Collection, and Identification of Plant Material

1.4.1 Selection

The selection of a suitable plant for an isolation of a new drug that is pharmacologically active is very important consideration. There are several ways for the selection of plants including traditional use, used of plants by the folk medicinal, used of plants for the isolation of drugs, chemical contents, toxicity, randomized selection or a combination of several criteria.

The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; this is known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. On the basis of above selection ways; I have selected one spice for this research purpose (Tyler *et al*, 1988).

1.4.2 Collection

Collection of drugs from cultivated plants always ensures a true natural source and a reliable product. This may or may not be the case when drugs are collected from wild plants. Carelessness or ignorance on the part of the collector can result in complete or partial substitution. This is especially true when drugs are difficult to collect or the natural source is scarce. Many drugs are collected from wild plants, sometimes on a fairly extensive scale (traga-canth, senna) when collection is the vocation of the gatherer, and sometimes on a limited scale when collection is an avocation (podophyllum, hydrastis). Because drugs come from all over the world, collection areas are almost universal, and collectors may vary from uneducated natives to highly skilled botanists.

The proper time of harvesting or collecting is particularly important because the nature and quantity of constituents vary greatly in some species according to the season. The most

advantageous collection time is when the part of the plant that constitutes the drug is highest in its content of active principles and when the material will dry to give the maximum quality and appearance (Tyler *et al*, 1988).

1.4.3 Harvesting

The mode of harvesting varies with each drug produced and with the pharmaceutical requirements of each drug. Some drugs may be collected by hand labor; however, when the cost of labor is an important factor, the use of mechanical devices is often more successful in economic production of the drug. With some drugs, where the skillful selection of plant parts is an important factor (digitalis), mechanical means cannot replace hand labor. The plant concerned to this research paper has been collected by hand labor (Sarker *et al*, 2005).

1.4.4 Drying

By drying the plant material, one removes sufficient moisture to ensure good keeping qualities and to prevent molding, the action of enzymes, the action of bacteria, and chemical or other possible changes. Drying fixes the constituents, facilitates grinding and Milling, and converts the drug into a more convenient form for commercial handling. Proper and successful drying involves two main principles: control of temperature and regulation of air flow.

Control of the drying operation is determined by the nature of the material to be dried and by the desired appearance of the finished product. The plant material can be dried either by the sun or by the use of artificial heat. With some natural products, such as vanilla, processes of fermentation or sweating are necessary to bring about changes in the constituents. Such drugs require special drying processes, usually called "curing." The plant of *opuntia elator* Mill has been dried by sun drying (Tyler *et al*, 1988).

1.4.5 Grinding

Small quantities of plant material can be Milled using a grinder or blinder or an electric spice Mill, or in a mortar and pestle. Milling of large quantities of plant material is usually best carried out using industrial-scale comminution equipment. Grinding improves the efficiency of

extraction by increasing the surface area of the plant material. It also decreases the amount of solvent needed for extraction by allowing the material to pack more densely.

Although it might seem that Milling plant material to a fine powder would be ideal, if the particles are too fine, solvent cannot flow easily around them. Furthermore, the friction of Milling generates heat (the finer the particle produced, the more heat), potentially causing volatile constituents to be lost, and thermolabile components to degrade and oxidize. Plants containing volatile components may be extracted by steam distillation of coarsely chopped plant material (Tyler *et al*, 1988).

1.4.6 Extraction of plant materials

For extraction, the plant materials should be properly authenticated. The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. Dried materials are powdered before extraction, whereas fresh plants (leaves, etc) can be homogenized or macerated with a solvent such as alcohol. Solvent such as alcohol is also useful for stabilizing fresh leaves by dropping them into the boiling solvent. Alcohol is a general solvent for many plant constituents (most fixed oils excepted) and as such may gives problems in the subsequent elimination of pigments, resins, etc. water immiscible solvents are widely used- Hexane, light petroleum(essential and fixed oil, steroids), ether and chloroform (alkaloids, quinones). The basification of the plants materials is required for the extraction of organic bases (e.g. alkaloids). For aromatic acids and phenols acidifications may be required. Extraction itself may be performed by repeated maceration with agitation, percolation, or by continuous extraction (e.g. in a Soxhlet extraction). Special methods for volatile oils (oils used in perfumery, such as oil of rose) are prepared by steam distillation, but many of the flower perfumes are extracted by enfleurage, by digestion in melted fats, by pneumatic methods or by means of solvents. In the enfleurage process, glass plates are covered with a thin layer of fixed oils or fat upon which the fresh flower are spread. The volatile oils gradually pass into the fat and the exhausted flowers are removed and replaced by a fresh supply. The pneumatic method, which is similar in principles to the enfleurage process, involves the passage of a current of a warm air through the flower. The air, laden with suspended flower oils, is then passed through a spray of melted fat until exhausted, when they are strained out and the perfume containing fat is allowed to cool. It will be seen that in each of the above processes the volatile oil has now been obtained in a fatty base.

The volatile oil is obtained from this by three successive extractions with alcohol. The alcoholic solutions may be put on the market as flower perfumes or the oil may be obtained in a pure form by recovery of the alcohol. Solvent extraction is based on the Soxhlet principle (Sarker *et al*, 2005).

1.4.7 Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted**.**

The various solvents that are used in the extraction procedures are:

a) **Water:** Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanin's) have no antimicrobial significance and water soluble phenolic only important as antioxidant compound (Das K *et al*, 2010).

b) **Acetone:** Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in

aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity (Eloff JN, 1998).

c) **Alcohol:** The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results (Lapornik B *et al*, 2005).

d) **Chloroform:** Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan MM, 1999).

e) **Ether:** Ether is commonly used selectively for the extraction of coumarins and fatty acids.

f) **Dichloromethanol:** It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only terpenoids.

1.5 Medicinal Plants:

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions,and to defend against attack from predators such as insects, fungi and herbivorous mammals. Any plant whose roots, leaves, seeds, bark, or plant part is used for therapeutic, tonic, purgative, or other health-promoting purposes. plants used as natural medicines.

Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: " A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesizing of useful drugs" (Sofowara, 1982).

Plants are valuable for modern medicine in four basic ways:

i. They are used as sources of direct therapeutic agents.

ii. They serve as raw materials base for elaboration of more complex semi synthetic chemical Compounds.

iii. The chemical structures derived from plant sources can be used as models for new synthetic compounds.

iv. Finally plants can be used as taxonomic markers for the discovery of new compounds (Reddy *et al*, 2010).

1.6 Humans and Plants

Humans need plants. All animals do. Humanity's relationship with plants has actually made it possible for us to have a civilization. Before we had cities, humans went around in little packs and were hunter-gatherers. We ate rats, birds, berries, and whatever food we could find. It wasn't very efficient. One day someone had the bright idea to plant the plants we like to eat. When

humans did that, they were able to stay in one place full time. Then came the cities and a huge system of agriculture to support Millions of people.

Everything we eat comes directly or indirectly from plants. Throughout human history, approximately 7,000 different plant species have been used as food by people. Plants regulate the water cycle: they help distribute and purify the planet's water. They also help move water from the soil to the atmosphere through a process called transpiration. Oxygen is brought to you by plants, as a byproduct of photosynthesis. Plants store carbon, and have helped keep much of the carbon dioxide produced from the burning of fossil fuels out of the atmosphere. Of course, aside from humans' myriad uses, plants make up the backbone of all habitats. Other species of fish and wildlife also depend on plants for food and shelter (Bgci.org, 2014).

1.7 Goals of Using Medicinal Plants as Therapeutic Agents

The goals of using plants as sources of therapeutic agents are – a) To isolate bioactive compounds for direct use as drugs, (E.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastine, Vincristine); b) To produce bioactive compounds of novel or known origin as lead compounds for semi synthesis to produce molecules of higher activity and / or lower toxicity, (E.g. Metformin, Nabilone, Oxycodone and other narcotic analgesics, Taxotere, Teniposide, Verapamil, and Amiodarone, which are based on Galegine, Δ9 – tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin respectively); c) To use agents as pharmacologic tools (E.g. LSD, Mescaline, Yohimbine); and d) To use the whole plant or part of it as a herbal remedy, (E.g. Cranberry, Echinacea, Feverfew, Garlic, Ginkgo biloba) (Fabricant, *et al,* 2001).

1.8 Importance of Medicinal Plants:

According to WHO, 80% people of the developing countries chiefly rely on traditional medicines involving the use of plant extracts or their active constituents. Only a portion of the plants of the world have been screened thoroughly for their medicinal value in order to find out newer plant derived drugs (Farnsworth, *et al,* 1991).

Plants have provided much life – saving pharmaceutical agents so far. And, there is an intense ongoing documentation of ethnomedical data and scientific research on medicinal plants by many developing countries. 14 of 35 in every 2000 drugs are either natural products or their

derivatives. The plants that are not studied phytochemically can thus provide potential new leads for newer drug development. For example, Galegine from the herb *Galegaofficinalis* was the lead compound for the development of Metformin used in the treatment of type 2 diabetes (Ahmed, 2011).

Others: Importance of medicinal plants

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.
- Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.
- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants (Britannica, 2013).

1.9 Classification of medicinal plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Joy *et al*, 1998).

Table 1.1: Classification of medicinal plants (Joy et al. 1998)

Based on parts used	1.whole pant: diffusa, *Phyllanthus neruri* *2.* Root: Dasamula 3. Stem:*Tinospora cordifolia, Acorus calamus* *4.* Bark: *Saraca asoca* *5.* Leaf: *Indigofera tinctoria, Lawsonia inermis,* Aloe vera 6. Flower:*Biophytum sensityvum, Mimusops elenji* *7.* Fruit: Solanum species 8. Seed: *Datura stramonium*

Based on habitat	1. Tropical: *Andrographis paniculata* 2. Sub-tropical: *Mentha arvensis* **3.** Temperate: *Atropa belladonna*

Based on therapeutic value	1. Antimalarial: *Cinchona officinalis, Artemisia annua* 2. Anticancer: *Catharanthus roseus, Taxus baccata* 3. Antiulcer: *Azadirachta indica, Glycyrrhiza glabra* 4. Antidiabetic: *Catharanthus roseus, Momordica charantia* 5. Anticholesterol: *Allium sativum* 6. Antiinflammatory: *Curcuma domestica, Desmodium gangeticum* 7. Antiviral: *Acacia catechu*

	8. Antibacterial: *Plumbago indica* 9. Antifungal: *Allium sativum* 10. Antiprotozoal: *Ailanthus sp., Cephaelis ipecacuanha* 11. Antidiarrhoeal: *Psidium gujava, Curcuma domestica* 12. Hypotensive: *Coleus forskohlii, Alium sativum* 13. Tranquilizing: *Rauvolfia serpentina* 14. Anaesthetic: *Erythroxylum coca* 15. Spasmolytic: *Atropa belladona, Hyoscyamus niger* 16. Diuretic: *Phyllanthus niruri, Centella asiatica* 17. Astringent: *Piper betle, Abrus precatorius* 18. Anthelmentic: *Quisqualis indica, Punica granatum* 19. Cardiotonic: *Digitalis sp., Thevetia sp.*

	20. Antiallergic: *Nandina domestica, Scutellaria baicalensis* 21. Hepatoprotective: *Silybum marianum, Andrographis paniculata*

1.10 Traditional Medicine

Traditional medicine (also known as indigenous or folk medicine) comprises knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called alternative medicine. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, Muti, Ifá, and traditional African medicine. Core disciplines which study traditional medicine include herbalism, ethnomedicine, ethnobotany, and medical anthropology (Singh *et al*, 2008).

The WHO notes however that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems. The line between alternative medicine and quackery is a contentious subject.

Traditional medicine may include formalized aspects of folk medicine, that is to say longstanding remedies passed on and practiced by lay people. Folk medicine consists of the healing practices and ideas of body physiology and health preservation known to some in a culture, transmitted informally as general knowledge, and practiced or applied by anyone in the

culture having prior experience. Folk medicine may also be referred to as traditional medicine, alternative medicine, indigenous medicine, or natural medicine. These terms are often considered interchangeable, even though some authors may prefer one or the other because of certain overtones they may be willing to highlight. In fact, out of these terms perhaps only indigenous medicine and traditional medicine have the same meaning folk medicine, while the others should be understood rather in a modern or modernized context (Shang *et al*, 2007).

Similarly, a home remedy is a treatment to cure a disease or ailment that employs certain spices, vegetables, or other common items. Home remedies may or may not have medicinal properties that treat or cure the disease or ailment in question, as they are typically passed along by laypersons (which have been facilitated in recent years by the Internet).

Among the largest ethnic group, the bangles on the main land, there are two distinct forms of Traditional medicine practice:

a) One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:

- **Folk medicine,** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting , bone-setting, , hot and cold baths, therapeutic fasting and cauterization.

- **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc.

- **Spiritual medicine,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

b) The other is the improved and modified form based on the following two main traditional systems:

- Unani-Tibb or Graeco-Arab system, which has been developed by the Arab and Muslim

scholars from the ancient Greek system, and

- Ayurvedic system, which is the old Indian system, based on the Vedas the oldest scriptures of the Hindu saints of the Aryan age (Ghani 1998).

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them.

1.11 Medicinal Plants in Bangladesh

In Bangladesh 5,000 species of angiosperm are reported to occur. The number of medicinal plants included in the *Materia Medica of Traditional Medicine* in this subcontinent at present stands at about 2,000. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. Dhaka, Rajshahi, Sylhet and Chittagong division is rich in medicinal plants where the cultivation of medicinal plants especially *Aloe vera* (Ghritakumari), *Asparagus racemosus* (Sotomuli), *Bombax ceiba* (Shimul), *Kaempferia angustifolia* (Misridana), *Ecolobium species* (Rajkantha) and *Ecolobium viride* (Nilkantha) are becoming popular.

Table 1.2: List of some Medicinal Plants used for Medicinal Purpose in Bangladesh

Scientific Name	Local Name	Traditional uses	Part(s) used
Bryonopsis laciniosa	Shivalingani	Skin Diseases, Dyspepsia, Jaundice	Whole Plant
Amorphophallus campanulatus	OlKachu	Piles, Tumors, Enlarged Spleen, Asthma, Rheumatism	Tuberous Roots

Hopea schaphula	Boilsur	Astringent, CNS depressant, Hypotensive	Stem Bark
Arachis hypogea	Cheenabadam	Emollient (Seeds), Bowel Astringent (Seeds Oil), Hemostatic Agent (Fruit Skin Extract)	Aerial Parts
Samanea saman	Fulkoroi	Diarrhea, Intestinal Diseases, Stomach Ache, Colds and Headache, Sore Throat	Bark
Michelia champaca	Champa	Fever, Colic, Leprosy, Post-Partum Protection, Eye Disorder	Seed and Flower
Aloe indica	Ghritakumari	Arthritis, hypertension Diabetes mellitus	Skin of Leaves
Swietenia mahagony	Mahagony	Diabetes, Malaria, Fever, Hypertension	Seeds

Caesalpinia nuga	Krung – khai	Analgesic, Anti Amyloidogenic, Antidiabetic / Hypoglycemic, Antifilarial, Anti-Inflammatory, Antimalarial, Antioxidant, Antitumor, Anxiolytic, Immunomodulatory.	Seed
Adansonia digitata	Baobab, Gadhagachh	Anti – malarial, Anti – pyretic, Anti – ulcerant, Health tonic	Leaf, Root, Flowe r
Jatropha gossypifolia	Karachuni, Bellyache Tree	Analgesic in toothache, Anti – diarrhoeal, Anti – malarial,	Leaf
Rauwolfia serpentina	Sharpagandha	Anti – hypertensive, Anti – malarial, Anti – psychotic	Root
Hodgsonia macrocarpa	Makal	Anti – malarial, Anti – pyretic	Fruit

1.12 Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in "Materiamedica" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants

majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (KritikarandBasu, 1980).

Bioactive compounds deposited in medicinal plants can serve as important raw materials for pharmaceutical manufacturing. Therefore, well-judged and scientific investigation of this wealth can significantly contribute to the public health. Again, it was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries. Thus, being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries (Chopra, *et al,* 1982).

1.13 **Economic Value**

Medicinal plants are good repository of bioactive compounds. They serve as important therapeutic agents as well as essential raw materials for the manufacture of traditional and modern medicines. They, therefore, play a vital role to constitute a precious natural wealth of a country and contribute a great deal to its health care program. A huge amount of foreign exchange can be earned by exporting medicinal plants to other countries. India and Thailand are two examples of such countries which earn a lot of foreign exchange by exporting medicinal plants and their semi-processed products to other countries including Bangladesh. In this way indigenous medicinal plants take part significantly to build up a healthy economy of a country.

1.14 Approaches of drug development

The major portion of the present day knowledge of the medicinal properties of plants is the sum total of some observations and experiences. According to some generous estimates, almost 80 percent of the present day medicines are directly or indirectly obtained from plants (Ghani 1998).

Steps of drug development from plant sources given below:

Selection of plant species:

- Preliminary screening of traditionally used plants
- Review literature and scientific result
- Authentication of data for their validity and comprehensiveness

Evaluation of toxicity:

- Gather data concerning toxicity and if demonstrate no toxicity then proceed to next step
- If toxicity data is not exit, select an appropriate test for toxicity analysis
- Develop and prepare bioassay protocol for safety and toxicity

Preparation of plant sample and element analysis:

- Collection of plant sample
- Extraction
    1. compare the selective and yield
    2. Use various extraction technique
- Analysis for elemental contents

Biological Testing:

- Selection of appropriate biological test
- Development protocol for biological test
- Analyze biological activity in- vivo
- Determine type and level of biological activity

Isolating active compounds:

- Isolating and characterization of compounds responsible for
- observed biological activity
- Evaluation of active compounds singularly and in combination with others to explore existence of activity and/or synergy of biological effect

In-vivo analysis:

- Use animal model for bioactivity analysis of active compounds
- Analyze again safety and toxicity but in in-vivo
- Conduct human studies

Commercialization:

- Develop appropriate dose delivery system
- Analyze cost-effectiveness
- Sustainable industrial production

1.15 Antioxidant activity

The main goal of antioxidant activity test is to find the oxidation- reducing power of the plant extract.

Oxidation in living organisms is essential for the acquirement of energy in catabolism. However, oxygen-centered free radicals and other reactive oxygen species, which are continuously, produced in vivo result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging, and diseases such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell & Gutteridge 1999).

Free radicals are natural by-products of human metabolism. These are charged molecules which attack cells, breaking cellular membranes and reacting with the nucleic acids, proteins, and

enzymes present in the cells. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and eventually result in cell dysfunction. They are continuously produced by our body's use of oxygen, such as in respiration and some cell-mediated immune functions. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution, pesticides, etc (Li & Trush 1994). Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant defense systems which scavenge these free radicals preventing them from causing deleterious effects in the body (Nose 2000). The antioxidant defense systems in the body can only protect the body when the quantity of free radicals is within the normal physiological level. But when this balance is shifted towards more free radicals, increasing their burden in the body either due to environmental conditions or infections, it leads to oxidative stress (Finkel & Holbrook 2000).

When the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system, oxidative stress occurs in cellular system, including the superoxide anion radical, the hydroxyl radical, hydrogen peroxide and the peroxyl are greatly reactive molecules, which consequently generate metabolic products that attack lipids in cel1 membrane or DNA (Halliwell & Gutteridge 1999). Oxidative stress, involves a series of free radical chain reaction processes, is associated with several types of biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging (Anderson et al. 2000). Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng et al. 1997). Improved antioxidant status helps to minimize the oxidative damage and thus can delay or decrease the risk for developing many chronic age related, free radical induced diseases (Karuna et al. 2009). The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years as the possibility of toxicity of synthetic antioxidants has been criticized. Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory,

antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent (Owen *et al*, 2000).

1.16 Plant review:

Scientific Name: *Opuntia elatior* Mill.

Figure1.1: *Opuntia elator* Mill plant

1.16.1 Plant description:

A spiny shrub with flattened, articulated, fleshy stems, up to 1.5 m high. Leaves 3.8 mm long, conical from a broad base. Joints 30-40 cm long, broadly obovate, not very thick, dull bluish-green. Aereoles large, bearing 4-6 prickles the largest very stout, subulate, sharp, 2.5-3.8 cm long. Flowers 7.5 cm across, yellow tinged with orange. Berry pyriform, truncate, depressed at the apex. Stems contain malate of manganese, a fatty acid, citric acid, wax, resin and sugar. Fruits contain carbohydrates (mucilage, sugars), albuminoids, fat, vitamin C and other fruit acids. Ripe fruits contain a red pigment, betanin. Flowers contain flavonoids, glycosides of *iso*-rhamnetin, quercetin, *iso*-quercitrin and narcissin. The plant also contains β-sitosterol, opuntiol and opuntiol acetate. A polysaccharide containing galactose and arabinose in 3:1 molar ratio has been isolated from pods (Rastogi and Mehrotra, 1990 & 93).

1.16.2 Taxonomy:

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Caryophyllales

Family: Cactaceae

Genus: Opuntia Mill

Species: Opuntia elatior Mill

1.16.3 Synonyms:

Arabic: Jhakawoon

Bengal: Negphana, Phanimansa

Burma: Kalzaw, Shasounglitwa

Canarese: Chappatigalli, Dabbugalli, Mullugalli, Nagadali, Papasakalli**,** Papasukattale, Sivaramakalli

Deccan: Chappal, Chappalsend, Nagphansi

English: Prickly pear, Slipper Thorn,

French: Raquette,

Gujarati: Chorhathalo, Zhorhatheylo

Hindi: Haththathoira, Nagphana, Nagphani

Malayalam: Nagamullu, Nagatali, Palakakkalli

Marathi: Chapal, Nagaphana Samar

Portuguese: Palmatoria d'inferno

Telugu: Nagadali, Nagajemudu, Nagamullu

Tulu: Kalli

Urdu: Nagaphani, Thuar

Uriya: Nagophenia, Nagopheni, Poturiyasiju

1.16.4 Traditional use of opuntia elator Mill:

The plant is digestive, carminative, diuretic and purgative; good for bronchitis of children, leucoderma, enlarged spleen, urinary burning, vesicular calculi and ophthalmia. Pounded plants are rubbed on scalp to clear dandruff. Leaves are used as a poultice to allay inflammation and heat; heated and applied to boils to hasten suppuration. Fruits are recommended as an expectorant and remedy for whooping cough, ashma and gonorrhoea (Yusuf *et al.* 2009). In Gujarat Opuntia elatior Mill., a member of Cactaceae family known as Hathlo-thore has been reported for its traditional uses as a medicinal plant. It is used as remedy in different disease conditions like anaemia, asthma etc. by tribal of Gujarat state. It is observed that the different species of Opuntia are highlighted for their ethno-medicinal claims like antibacterial, anti-diarrheal, anti-inflammatory, analgesic, anti-spermatogenic, anti-microbial and anti-diabetic properties (Mpbd.info, 2015).

1.17 Aim of this experiment

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products.

Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs ,problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity.

The main objective of this study was to discovery of new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Opuntia elator Mill is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on this plant. The principal aim of the present study was to investigate the scientific basis of the traditional uses of the plant the crude extract of *Opuntia elator Mill* to evaluate their in- vitro pharmacological activities.

Chapter: Two

LITERATURE REVIEW

2. Literature review of the biological activities of *Opuntia elator* Mill

2.1 Analgesic and Anti-inflammatory action of *Opuntia elatior* Mill fruits

The fruits of *Opuntia* produced analgesic and anti-inflammatory action in a dose-dependent manner. ED_{50} values of the fruit juice in writhing, tail immersion, and paw edema test were 0.919, 2.77, and 9.282 ml/kg, respectively. The OFJ (20 ml/kg) showed no significant change in various autonomic and behavioral responses of rat compared to the control animals. No mortality was recorded until 48 h in the animals treated with OFJ up to 20 ml/kg oral dose, and therefore, considered to be safe. The dose of OFJ was selected after carrying out acute toxicity studies. According to the finding, the maximum dose (20 ml/kg) was safe and based on that we selected three different doses for the study: low (5 ml/kg), medium (15 ml/kg), and high (15 ml/kg). The phytochemical analysis indicated the presence of color pigment betacyanin as the active principle, in addition to being a good source of sugar content and low acidity which make the fruit sweet and delicious. The total betacyanin content (47.1 mg/100 ml) equivalent to betanin obtained from the fruits of *O. elatior* Mill. was higher than that in *O. ficus-indica* and *O. undulate* Griff., while it was lower than that in *O. stricta* Haw.

In control animals, the subplantar injection of carrageenan produced a local edema that increased progressively to reach the maximum intensity at 5 h after the injection of the phlogistic agent (90.68 ± 4.21%). OFJ at oral doses of 10 ml/kg and 15 ml/kg showed significant suppression of carrageenan-induced rat paw edema after 2 h when compared with the control group ($P < 0.01$ and $P < 0.001$, respectively). Diclofenac sodium also showed a clear inhibition of the inflammation induced by carrageenan after 2 h when compared with the control group ($P < 0.001$). Pretreatment by OFJ significantly reduced ($P < 0.001$) the carrageenan-induced edema in a dose-dependent manner, 3 h after carrageenan injection, to reach the maximal inhibition at this time with the dose of 15 ml/kg (54.69 ± 5.98%) and ED_{50} at 9.282 (Chauhan *et al*, 2015).

2.2 Effect of fruits of *Opuntia elatior* Mill on mast cell degranulation

In the study, the fruit juice of *O. elatior* Mill. was found to inhibit the degranulation of mast cells induced by an immunological and a nonimmunological stimulus.Effect of Opuntia fruit

juice on compound 48/80 induced rat mast cell degranulation.The phytochemical analysis indicated the presence of color pigment betacyanin as an active principle and a good source of sugar content and low acidity of the fruit that make it very sweet and delicious. The total betacyanin content (47.1 mg/100 ml) equivalent to betanin obtained from fruits of O. elatior Mill. was higher when compared to O. ficus-indica and Opuntia undulate Griff. while lower when compared to O. stricta Haw.

The OFJ (10-200 µl/ml) were studied for the effect on sensitized rat peritoneal mast cell degranulation induced by immunological (egg albumin) and nonimmunological (compound 48/80) stimuli and compared with that of the reference compound (sodium cromoglycate and ketotifen, 10 µg/ml). Egg albumin and compound 48/80 incubated mast cell demonstrated 93.17% and 92.63% of degranulation, respectively. Sodium cromoglycate and ketotifen as a reference standard produced an inhibition of 75.33% and 80.33% in egg albumin and 79.33% and 84.33% in compound 48/80–induced degranulation, respectively. The OFJ exhibited significant ($P < 0.001$) concentration – dependent inhibition of mast cell degranulation with respect to positive control. Further, we observed better inhibition with OFJ (200 µg/ml) than sodium cromoglycate in egg albumin induced mast cell degranulation. The IC_{50} of fruit juice was 12.24 µg/ml and 18 µl/ml for egg albumin and compound 48/80 induced mast cell degranulation, respectively (Chauhan *et al*, 2015).

2.3 An Active Principle of *Opuntia elatior* Mill as an Eco-Friendly Inhibitor of Corrosion of Mild Steel in Acid Medium

The anticorrosion ability of *Opuntia elatior* fruit extract was tested on mild steel (MS) in 1 M HCl and H_2SO_4 media by a weight loss method at various temperatures, electrochemical experiments such as potentiodynamic polarization (PDS) and electrochemical impedance spectroscopy (EIS), and surface characterization techniques using scanning electron microscope (SEM) and X-ray diffraction (XRD) studies. The major phytoconsituent, opuntiol, was isolated chromatographically and characterized by infra-red (IR) and nuclear magnetic resonance (NMR) spectroscopic studies. Further, its corrosion inhibitive effect was investigated by PDS, EIS, SEM, and XRD studies. The results of the weight loss studies indicated that inhibition efficiencies were enhanced with an increase in concentration of extract and decreased with a rise

in temperature. Adsorption of the extract on a mild steel surface obeyed the Temkin isotherm. Results of PDS revealed the mixed mode inhibitive action, and results of EIS studies confirmed the adsorption of the extract at the metal–solution interface. Further, SEM and XRD studies clearly revealed the film-forming ability of opuntiol on the surface of mild steel. Thus, the anticorrosion activity of *O. elatior* can be related to the presence of opuntiol (Loganayagi *et al*, 2014).

2.4 Reversible antifertility effect of *Opuntia elatior* Mill. Fruit extract

This study was undertaken to evaluate the male antifertility potential of the fruit extract of O. elatior and its reversibility. The methanol extract of the fruit of O. elatior shows reversible male antifertility activity without affecting the serum testosterone levels and libido. Epididymal sperm count and motility was markedly reduced up to 75-80% in rats treated for 60 days, without commensurate decline in serum testosterone levels. Testicular steroidogenesis was not affected as evident by the hydroxysteroid dehydrogenase activity. Fertility of the treated rats was suppressed when mated with normally cycling virgin female rats without affecting libido. The fertility suppression was dose-dependent being 100% in the highest dose. Withdrawal of the treatment for two weeks led to recovery of the epididymal sperm count, testicular HSDH activity, serum testosterone levels and the fertility (MC, Shivabasavaiah, 2015).

2.5 Ethnomedicinal value of *Opuntia elatior* Mill fruits and its effects in mice:

Opuntia elatior Mill belongs to Cactaceae family is an important plant used as medicine for various ailments due to beneficial health promoting properties. The present study was undertaken to evaluate the nutritional components present in the cactus pear fruit. The ripened fruits of *Opuntia elatior* Mill were collected, authenticated, air dried, powered and subjected for ethanol extraction. The fruit extract was screened for its phytochemical components, which revealed the presence of alkaloids, carbohydrates, fats, oils, flavonoids, phenolics, tannins, steroids, and saponins. The oral administration of crude extract exhibited no toxic effect on the external morphology and body weight of the mice. Thus, the present investigation established scientific base for further use of *Opuntia elatior* for various pharmacological tests like antibacterial, antidiarrheal, anti-inflammatory, analgesic, antispermatogenic, antimicrobial and antidiabetic properties. The phytochemical research based on ethnopharmacological information

is generally considered as an effective approach in the discovery of new anti infective agents, hence this study focused on the detection of phytochemical compounds present in *Opuntia elatior*fruits and its toxicity (Anon, 2015).

2.6 Antioxidant potential and its relationship with polyphenol content and degree of polymerization in *Opuntia elatior* Mill. Fruits:

Opuntia elatior Mill. (*Nagaphani*) fruits are traditionally recommended as an expectorant, remedy for whooping cough, asthma, gonorrhea, ulcers, tumors, in the treatment of diarrhea and syphilis. Many of these diseases are allied with oxidative stress caused by free radicals. Thus, current research is directed towards finding naturally-occurring antioxidants of plant origin. The experimental data indicated that the HAOE, ethyl acetate (EAOE) and butanol (BFOE) soluble fractions have shown significant antioxidant activity. The highest polyphenolic, FA, FO contents and degree of polymerization were found in EAOE. The scavenging potential was in the order of Ascorbic Acid > EAOE > BFOE > HAOE > BIOE, where ascorbic acid was used as a positive control. The increased antioxidant potential of EAOE and BFOE fractions over HAOE extract may be attributed to the purification achieved by fractionation of the extract which in turn resulted in an increase in the degree of polymerization and segregation of secondary metabolites (Itankar *et al*, 2014).

2.7 Drought-stress-induced up-regulation of CAM in seedlings of a tropical cactus, *Opuntia elatior* Mill, operating predominantly in the C3 mode

Immediately after unfolding, cotyledons of the tropical platyopuntoid cactus, *Opuntia elatior* Mill., exhibited a C_3-type diel CO_2 exchange pattern characterized by net CO_2 uptake in the light. Significant nocturnal increases in titratable acidity typical of crassulacean acid metabolism (CAM) were not detected at this early developmental stage. As cotyledons matured and the first cladode (flattened stem) developed, features of CAM were observed and the magnitude of CAM increased. Nonetheless, in well-watered seedlings up to 10 cm tall, C_3 photosynthetic CO_2 fixation in the light remained the major pathway of carbon fixation. Reduced soil water availability led to an up-regulation of net dark CO_2 fixation and greater nocturnal increases in tissue acidity, consistent with facultative CAM. These observations demonstrate that C_3 photosynthesis, drought-stress-related facultative CAM, and

developmentally controlled constitutive CAM can all contribute to the early growth of *O. elatior*. The strong C_3 component and facultative CAM features expressed in young *O. elatior* contrast with mature plants in which obligate CAM is the major pathway of carbon acquisition (Winter *et al*, 2011).

2.8 Phytochemical study and evaluation of antileukemic activity of ripe fruit of *Opuntia elatior* Mill:

The preliminary phytochemical evaluation showed the presence of carbohydrates, flavonoids, betanin pigment, tannins, sterols, proteins, pectin, citric acid and vitamin C. The total phenolic, flavonoid, flavonone and betanin content in extract were found to be 52.76 mg/g, 39.22 mg/g, 9.60 mg/g and 48.10 mg/100ml of extract respectively. Evaluation of antioxidant activity was carried out by DPPH free radical scavenging method. It showed significant activity compared with the standard. IC50 values for extract of O.elatior and Vitamin C was found to be 88.16 g/ml and 62.83 g/ml respectively (Itankar *et al.*, 2012).

2.9 Evaluation of bronchodilatory properties of fruits of *Opuntia elatior* Mill

Traditionally, fruits of *Opuntia elatior* Mill. were used for their immunomodulatory and antiasthmatic action. The presence of potentially active nutrients and their multifunctional properties make prickly pear a perfect candidate for the production of phytopharmaceutical products. This study suggested that fruits of *O. elatior* Mill. possess a significant inhibitory effect on rat and guinea pig ileum, and betacyanin, an active principle compound in prickly pear, may be responsible for the action. Bronchodilating effect of fruit juice was dose dependent against spasm induced by acetylcholine and histamine. *O. elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction through antihistaminic and antimuscarinic action (Chauhan *et al*, 2015).

2.10 Haematinic Evalution of Fruits of *Opuntia elator* Mill on Mercuric chloride induced Anemia in Rats

The fruits of *Opuntia elatior* Mill. (Family: Cactaceae) is known as prickly pear and widely used in several indigenous systems of medicine for the treatment of various ailments, *viz.*

Anemia, asthma, inflammatory disorders, and diabetes. The objective of the present work is to screen phytochemical compositions and evaluation hematinic activity of fruits of *Opuntia elatior* Mill. The hematinic activity of an orally administered fruit juice (5, 10 and 15 ml/kg) was studied on mercuric chloride ($HgCl_2$) -induced anemic rats. Phytochemical analysis signifies the presence of betacyanin as an active principle which was confirmed by spectrophotometric, HPLC and LC-MS techniques. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from the fruits of *O. elatior* Mill. was higher compared to *O. ficus-indica* and *O. undulata* Griff. while lower compared to *O. stricta* Haw. Mercuric chloride altered the hematological parameters by hemolysis characterized by decrease in Hb content, total RBC counts and PCV ($p < 0.001$) on day 30. Fruit juice at the dose of 10 ml/kg and 15 ml/kg showed a good percentage of recovering in hemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating the correction of anemia induced by mercuric chloride after 30 days treatment. The speedy and progressive recovery of anemia in the treatment of prickly pear may be due to increased erythropoiesis and/or antioxidant property of betacyanin (Chauhan *et al*, 2014).

2.11 Evaluation of physicochemical property and fatty acid composition of *Opuntia elatior* seed oil:

Seed oil extracted from *Opuntia elatior* was analyzed for its physicochemical properties such as acid value, iodine value, saponification value, unsaponifiable matter and fatty acid composition. The assessment of fatty acid composition using gas liquid chromatography shows the presence of 15.65% saturated fatty acids and 84.35% unsaturated fatty acids. Linoleic acid was the dominating fatty acid followed by oleic and palmitic acids respectively. The oil analysis showed that the Iodine value, Acid value, Saponification value and Unsaponifiable matter were 110.95, 1.64, 191.85 and 2.65 respectively. *Opuntia elatior* appears to be a good potential source of seed oil for industrial utilization.

2.12 Histochemical studies in stomatal apparatus of Phaseolus mungo Linn, Lathyrus sativus Linn and *Opuntia elatior* Mill

With histochemical methods the distribution of some enzymes and metabolic substances in the epidermal peelings of Phaseolus mungo, Lathyrus sativus, and *Opuntia elatior* under light and dark conditions is examined. Dehydrogenases oxidases, transferases and hydrolases were studied. Fluctuations in the activity of hydrolases, especially, acid phosphatase, lipase, glucose-6-phosphatase, adenosine triphosphatase, dehydrogenases and transferases were observed during light and dark conditions. The role of such fluctuations in relation to stomatal regulation is discussed. Based on the present studies the following is suggested; stomatal opening and closing is related to structural and metabolic changes, and these changes are brought about by sugar gradients in the guard cells; light is enhancing the synthesis of sugars and some hormones, and besides this it stimulates membrane bound adenyl cyclase and release of cyclic AMP which affects the permeability; subsidiary cells actively participate in the stomatal physiology. Lysosomal hydrolytic enzymes likeacid phosphatase are actively involved in catabolic phase of normal guard cells metabolism and regulate the osmotic pressure of the guard cells.(Chauhan, Sheth.2013).

2.13 Anti-ulcer activity of ethanolic extract of stem of *Opuntia elatior* Mill

Stem powder successively extracted with alcohol and was subjected for phytochemical screening to identify different phytoconstituents. Anti ulcer activity was evaluated by ethanol induced gastric ulcer in albino rats. Preliminary phytochemical studies revealed the presence of flavonoids, tannins, carbohydrates and proteins in alcoholic extract of O.elatior. Further alcoholic extract at 100, 200 and 400 mg/kg, p.o doses significantly ($P < 0.01$) reduced the ulcer score, ulcer number, ulcerindex, free acidity and total acidity in ethanol induced ulcer model in rats. The present study revealed the antiulcer activity of stem extract of O.elatior is due to the presence of flavonoid which is the cytoprotective active material for which antiulcerogenic efficacy has been extensively confirmed.

Chapter: Three

METHODS AND MATERIALS

3.1 plant collection, processing powdering, extraction, and filtration

3.1.1 Collection:

Opuntia elator Mill is not so available throughout the country. The plant is cactus species and it is found in nursery .The plant collected from Noakhali area. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days.

3.1.2 Process of powdering:

At first the plants were cleaned to remove dust, soil etc within them. After this the whole amount of plant was dried. The dried plants were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction.

The amount of powder was 800g. During powdering of sample, the blander was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blander.

3.1.3 Extraction:

The fine powder of plants was dissolved in 2 liter methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.4 Filtration:

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a conical flask and covered with

aluminum foil paper was prepared for rotary evaporation.

3.1.5 Evaporation and extract preparation:

For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reused it. For this solvent almost 70% solvent get back into liquid form. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

Figure 3.1: Drying of extract using rotary evaporator

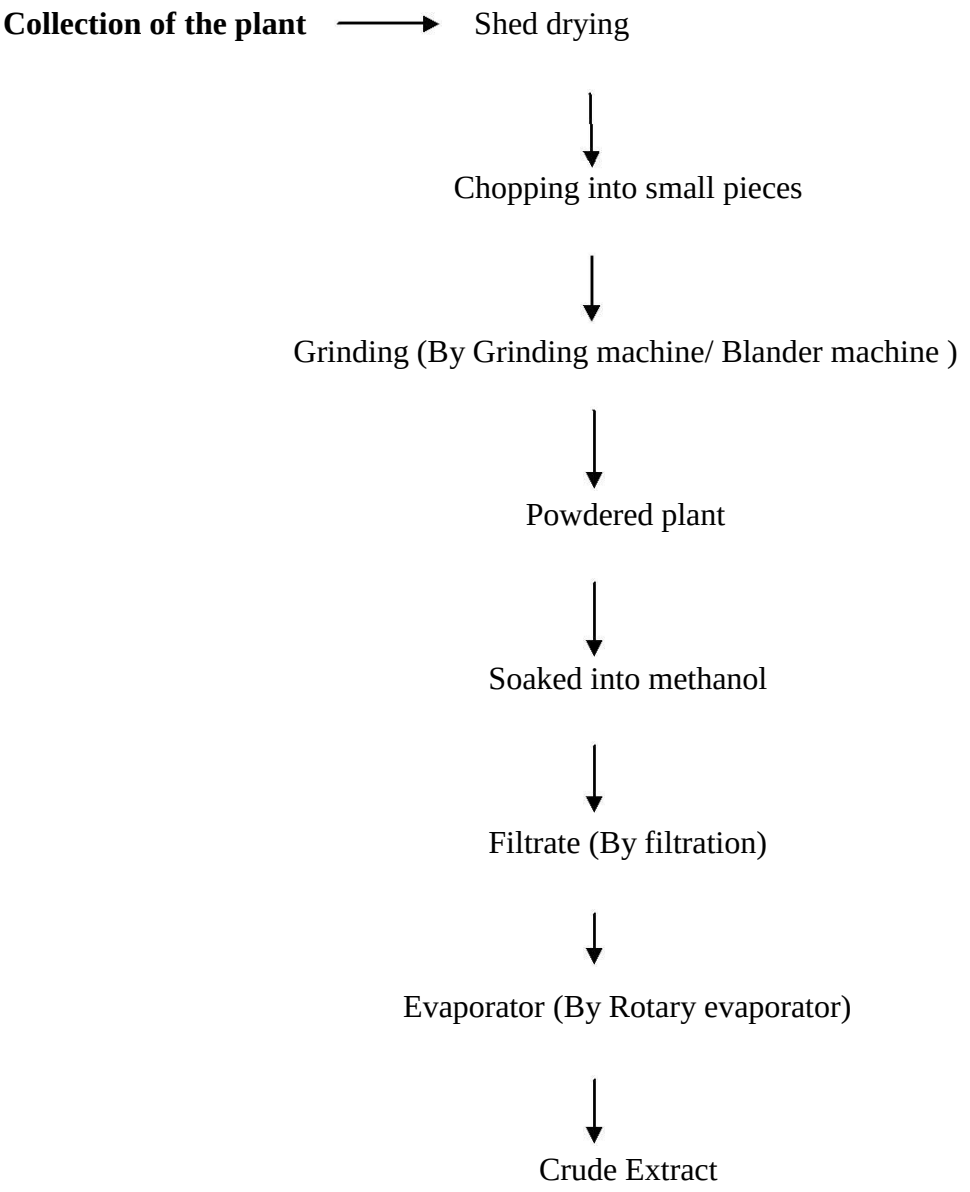


Figure 3.2: Schematic presentation of the crude preparation from the plant

3.2 Partition of mother solution

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

3.2.1 Partition with pet-ether

The mother solution was taken in a separating funnel. 100ml 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 (100ml × 3). The pet-ether fraction was then air dried for solid

residue.

3.2.2 Partition with DCM

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

3.2.3 Partition with ethyl acetate

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

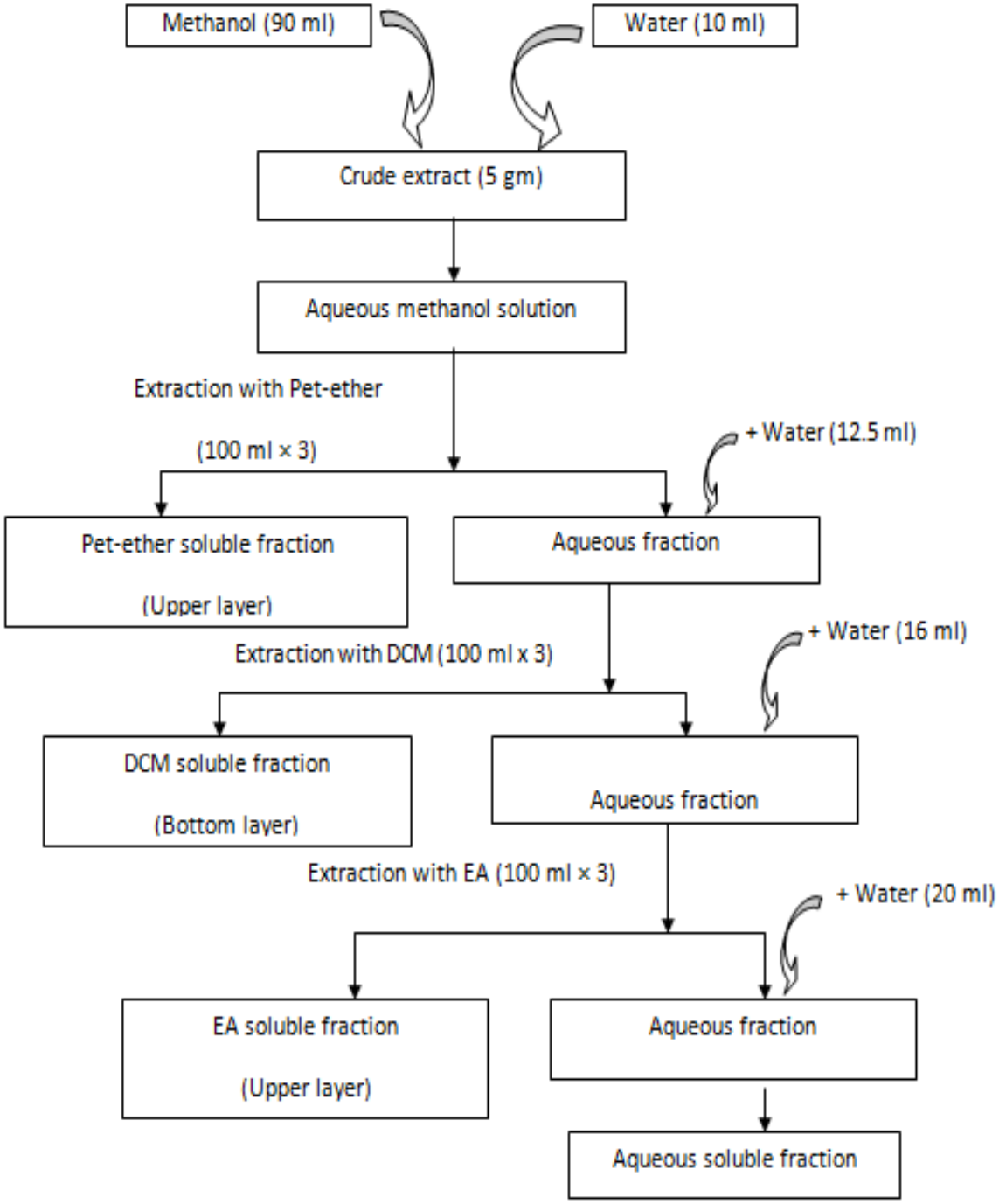


Figure 3.3: Schematic representation of the partitioning of methanolic crude extract of *Opuntia elator* Mill

3.2.4 Collection Of crude extract

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

3.3 Antioxidant activity

3.3.1 Total Phenolic Content

The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi, Janitha, &Wanasundara, 1992). 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 antioxidativestatus occurrence of human diseases (Rice,Evans, Sampson, Bramley, & Holloway,1997). 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 (Rimbach,&Virgili, 1999). 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 *Ficus racemosa*-as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity.

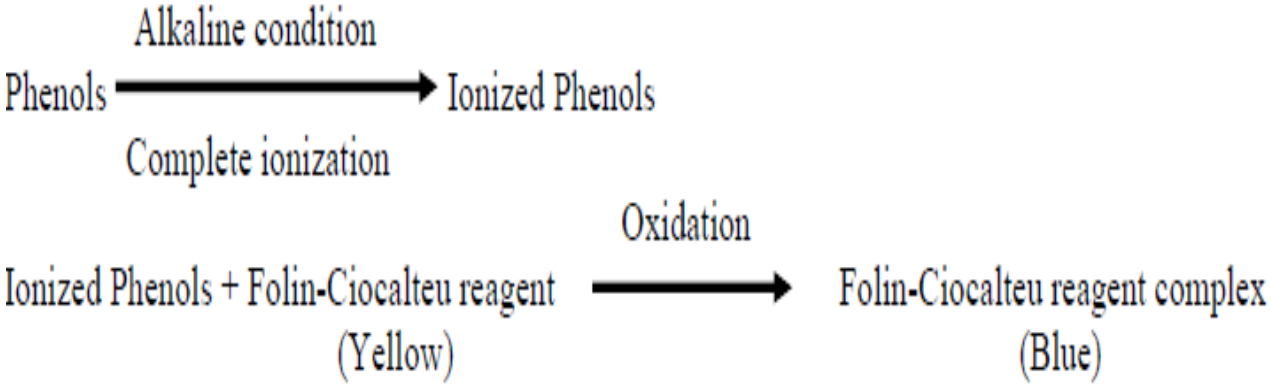
3.3.2 Principle

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 100mg Folin-Ciocalteu Reagent

Ingredient	**Amount**
Water	57.5ml
Lithium Sulfate	15.0mg
Sodium Tungstate Dihydrate	10.0mg
Hydrochloric Acid>=25%	10.0mg
Phosphoric Acid 85% solution in water	5.0mg
Molybdic Acid Sodium Dihydrate	2.5mg

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton *et al.*, 1999; Vinson *et al*, 2005).



3.3.3 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 & 200ml)
Na_2CO_3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200µl)
Distilled water	Cuvette

3.3.4 Procedure

3.3.4.1 Standard curve preparation:

Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120µg/ml to 80µg/ml. 5ml of FCR (diluted 10 times with water) and4ml of Na_2CO_3 (7.5%w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

3.3.4.2 Sample preparation:

2mg of the *Opuntia elator* Mill crude extract was taken and dissolved in 1ml of distilled water to get a sample concentration of 2mg/ml.

3.3.5 Determination of total phenol content:

- 1.0ml of plant extract (200µg/ml) of different concentrations (120µg/ml, 110µg/ml, 100µg/ml, 90µg/ml and 80µg/ml) was taken in test tubes.
- 5ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
- 4ml of Sodium carbonate solution was added into the test tube.
- The test tubes containing the samples were incubated for 1hr at the room temperature to complete the reaction.

- Then the absorbance of the solution was measured at 765nm using a spectrophotometer against blank.
- A typical blank solution containing methanol was taken.

3.4. Total Flavonoid Content

3.4.1 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 Aor B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorptivity maximum at 510nm (Chang *et al.*, 2002). Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard (Chang *et al*, 2002).

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

3.4.2. 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

3.4.3 Procedure

Preparation of 10% Aluminium Chloride ($Alcl_3$) Solution: 10mg of $AlCl_3$ was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

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

Preparation of 5% (W/V) $NaNO_2$ Solution: 5mg of $NaNO_2$ was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of Standard Solution: The stock solution was prepared by taking 0.025gm of ascorbic acid and dissolved into 5ml of ethanol. The concentration of this solution was 5µg/µl of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.4: Preparation of standard solution

Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by ethanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

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

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

For blank solution 1.5ml methanol was taken and the same procedure was repeated. Then the absorbance of the solution was measured at 510nm using a spectrophotometer against blank.

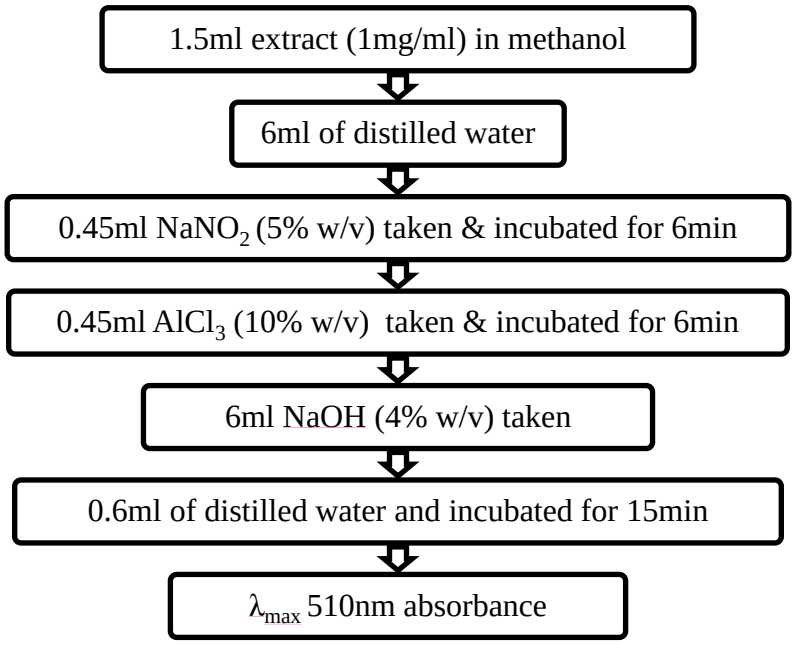


Figure 3.4: Schematic diagram of preparation of extract solution

Preparation of blank solution:

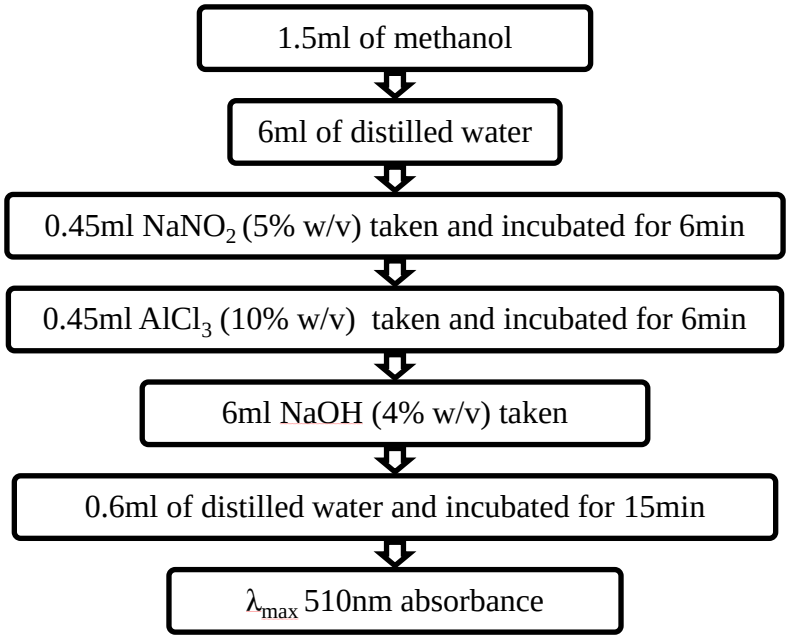


Figure 3.5: Schematic diagram of preparation of blank solution.

3.5 Reducing power assay

3.5.1Principle

The reducing power of crude extract of *Opuntia elatior* Millwas determined by the method of Oyaizu. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + ferrous chloride

3.5.2 Apparatus and reagents

- ✓ Potassium ferricyanide
- ✓ Phosphate buffer
- ✓ Trichloro acetic acid
- ✓ Ferric chloride
- ✓ Ascorbic acid
- ✓ Methanol
- ✓ Spatula
- ✓ Analytical balance
- ✓ Pipette and pumper
- ✓ Aqueous fraction
- ✓ Test tubes and beaker

3.5.3 Procedure

Phosphate buffer (0.2 M, pH 6.6) preparation

Dibasic sodium phosphate (18.75 ml of 0.2M) is mixed with 31.25 ml monobasic sodium phosphate and diluted to 100 ml with water.

Potassium ferricyanide (1% w/v) preparation

1mg of potassium ferricyanide ($K_3[Fe(CN)_6]$) was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

Trichloro acetic acid (10%) preparation

10mg of trichloro acetic acid (CCl_3COOH) was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

Ferric chloride (0.1%) preparation

0.1mg of ferric chloride ($FeCl_3$]) was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

3.5.4 Standard solution preparation

The stock solution was prepared by taking 0.025gm of ascorbic acid and dissolved into 5ml of methanol. The concentration of this solution was 5µg/µl of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.5: Different concentrations of ascorbic acid solution preparation

Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

3.5.5 Extract solution preparation

5mg of plant extract was taken and dissolved into 5ml of methanol. The concentration of the solution was 1mg/ml of plant extract.

3.5.6 Determination of reducing power

2.0 ml plant extract solution and ascorbic acid in different concentrations were taken in test tubes and mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 500°C for 20 min. 2.5 ml Trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5 ml upper layer (supernatant solution) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract.

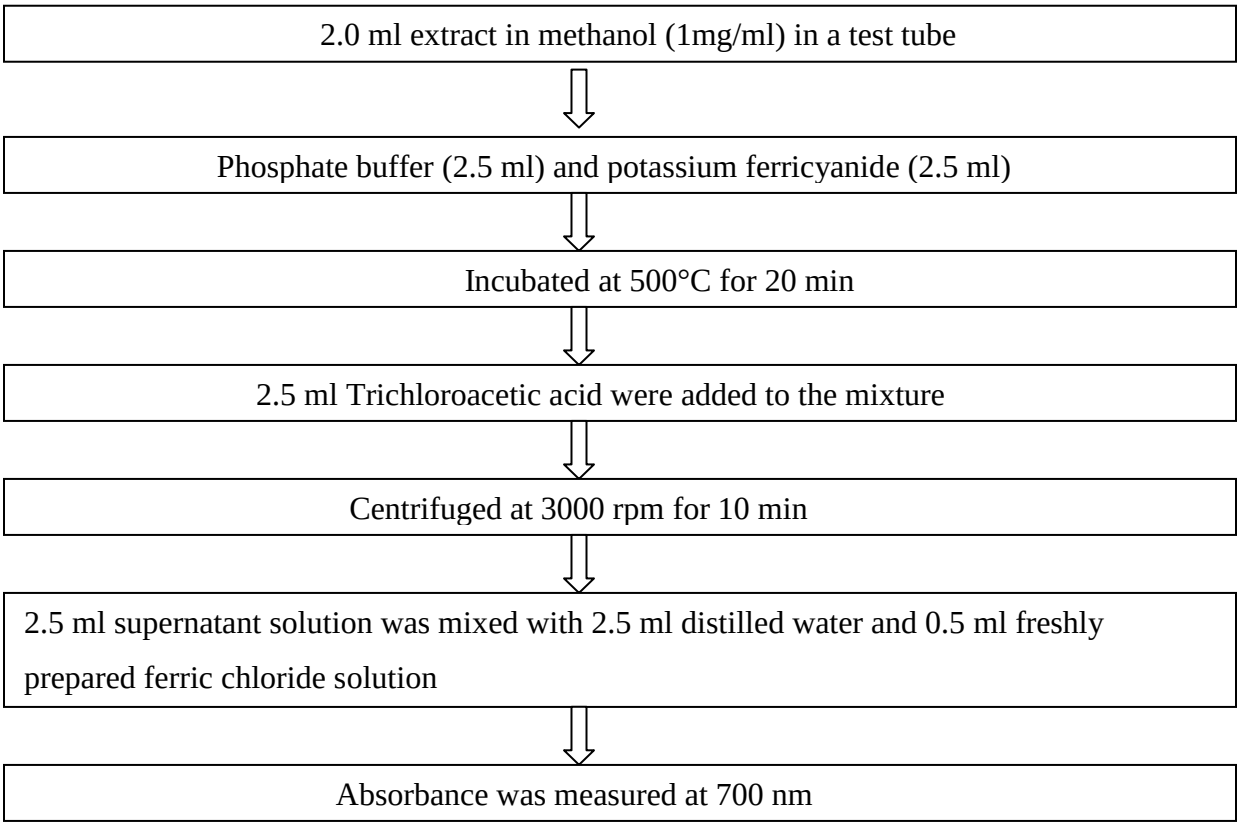


Figure 3.6: Schematic diagram of *Opuntia elator* Mill plant total reducing power

3.5.7 Calculation of total reducing power:

$$\%\,\text{increase in Reducing Power} = \left(\frac{A_{test}}{A_{blank}} - 1\right) \times 100\%$$

Where A_{test} is absorbance of test solution; A_{blank} is absorbance of blank. Increased absorbance of the reaction mixture indicates increase in reducing power.

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- *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 for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae (Olowa and Nuneza, 2013; Rishikesh *et al.*, 2013).

3.6.2 Apparatus & Reagents

Table 3.6: 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

3.6.3 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 *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 *Artemia salina* were hatched at room temperature (25-30ºC) for 18-24hr. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay.

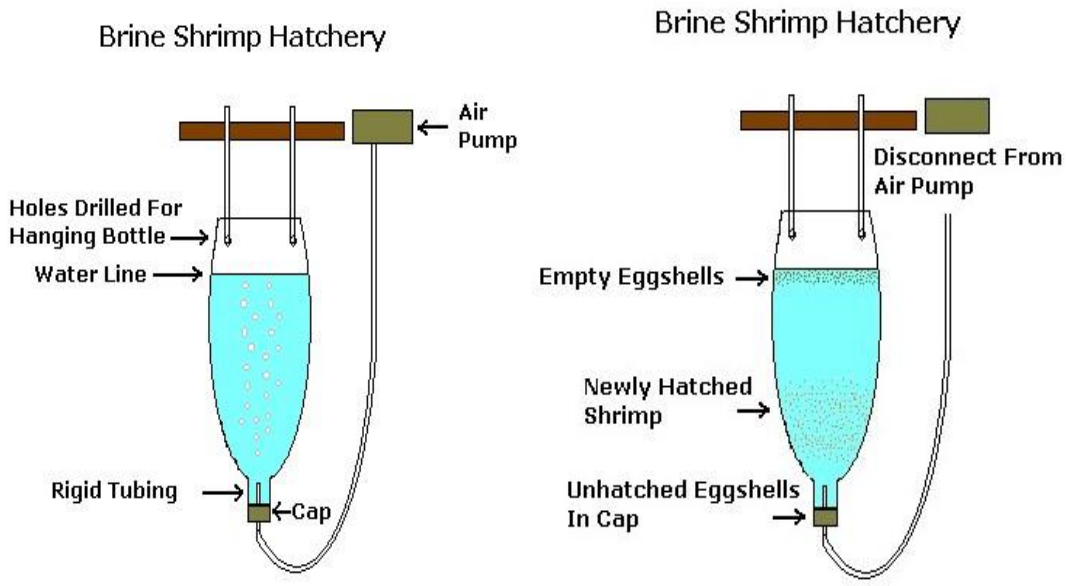


Figure 3.7: Brine shrimp Hatchery

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 Preparation of the Test Samples Of Experimental Plant

The crude extract 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.

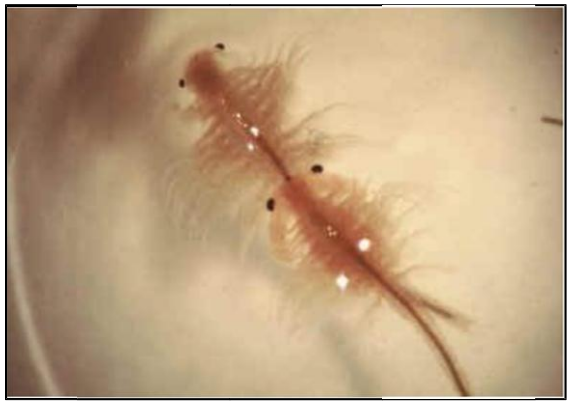


Figure 3.8: *Artemia salina* 24 hours old

Chapter: Four

RESULTS AND DISCUSSION

4.1 Antioxidant Test Results

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

- Determination of total phenolic content
- Determination of total flavonoids content
- Determination of total reducing power content

4.1.1 Result of Total Phenolic Content

The crude extract of *Opuntia elatior* Mill crude was subjected to determine total phenolic content. Ascorbic acid was used as reference standard.

4.1.2 Preparation of Standard Curve

Table 4.1 Total phenol content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 765 nm)**	**Regression line**	**R^2 value**
80	2.406	y = 0.0193x + 0.8246	0.9372
90	2.473		
100	2.767		
110	3.057		
120	3.080		

A linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.1. This linear curve was considered as a standard curve.

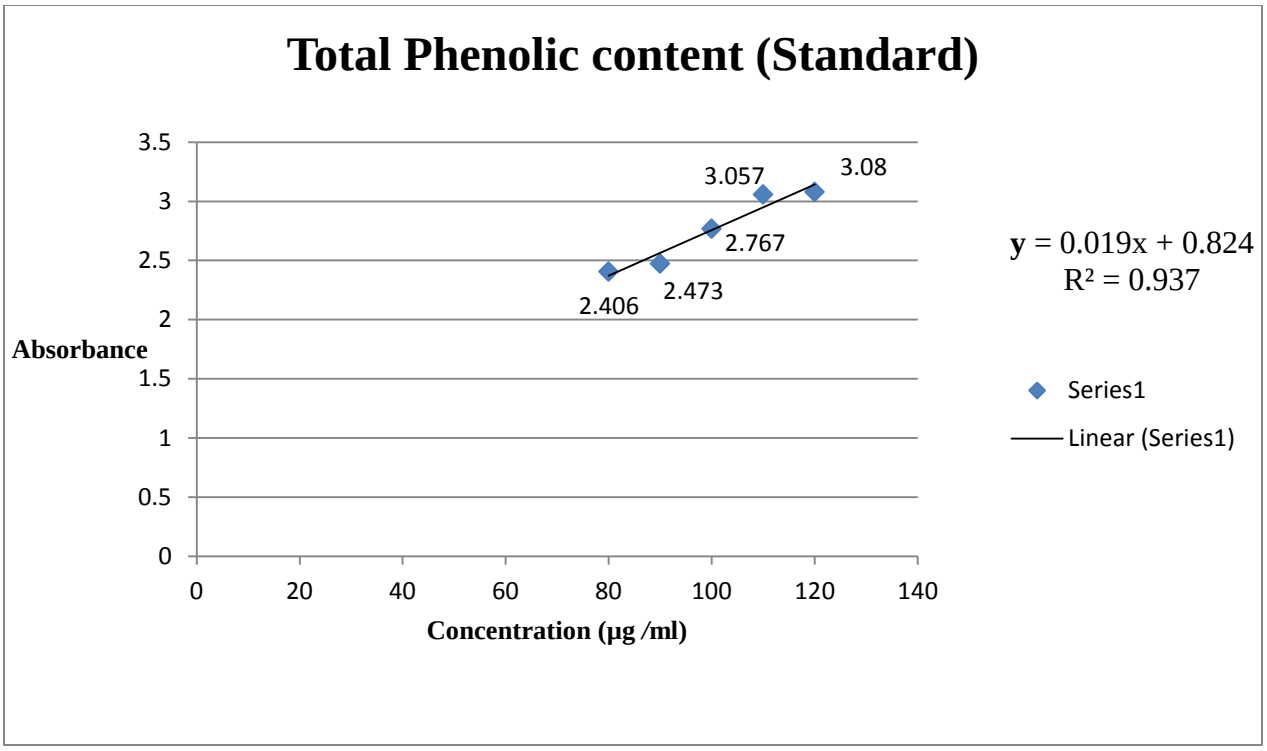


Figure 4.1: Graphical representation of assay of phenolic content of ascorbic acid

4.1.3 Total Phenol Content Present in crude Extract

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

Table 4.2 Total phenolic content of *Opuntia elatior* Mill crude extract

Concentration (mg/ml)	**Absorbance**	**X value (mg of AAE/gm of dried extract)**
2	1.732	23.895

4.1.4 Discussion

The absorbance was found to be directly proportional to the concentration in standard and crude extract samples. In crude extract and standard the absorbance increased with the increase in concentration indicating increase in phenolic content. Compare with the standard absorbance of

the extract is less than the standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 23.895 mg of AAE/gm of dried extract of phenol content was found in the *Opuntia elatior* Mill crude extract.

4.2 Result of Total Flavonoid Content

The *Opuntia elatior* Mill crude extract were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard.

4.2.1 Preparation of Standard Curve

Table 4.3: Total flavonoid content of ascorbic acid

Concentration (µg/µl)	**Absorbance (At 510 nm)**	**Regression line**	**R^2 value**
50	0.05	y = 0.0017x-0.042	0.991
100	0.13		
150	0.19		
200	0.29		
250	0.39		

After absorbances were taken of different solution of ascorbic acid of concentrations ranging from 50µg/µl to 250µg/µl, a linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.10 This linear curve was considered as a standard curve.

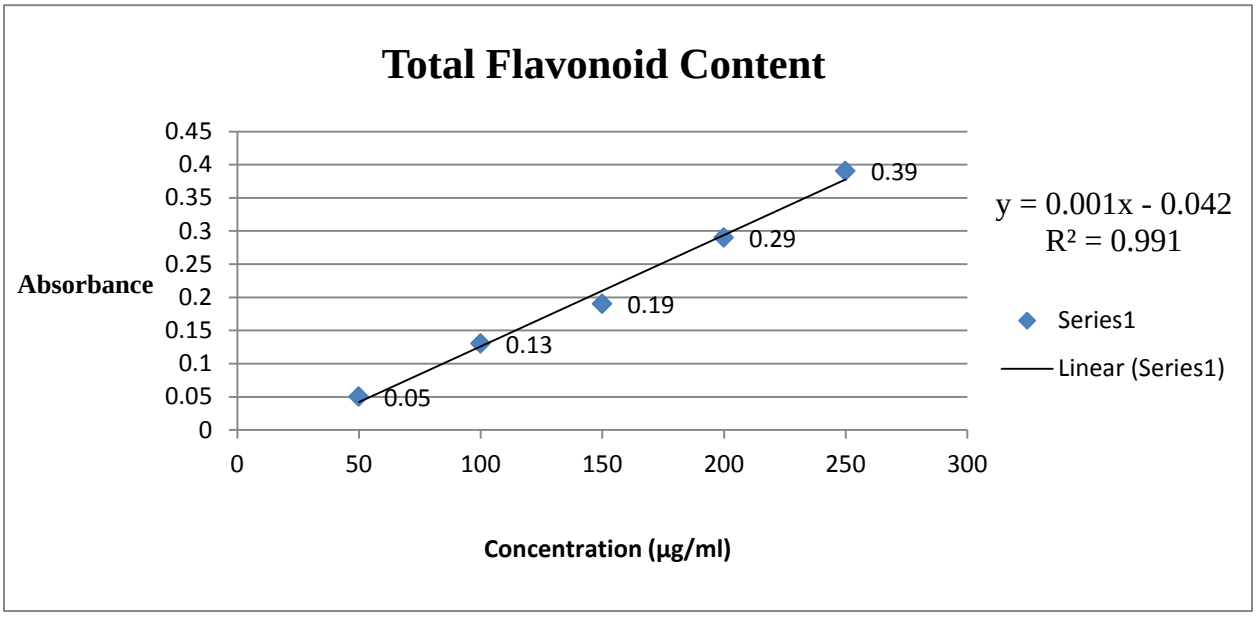


Figure 4.2 Graphical representation of assay of flavonoid content of ascorbic acid

4.2.2 Total Flavonoid Content Present in crude Extract

Based on the absorbance value of crude 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 4.4.

Table 4.4 Total flavonoid content of *Opuntia elatior* Mill crude extract

Sample	**Concentration (mg/ml)**	**Absorbance**	**Total flavonoid content (mg of AAE/g of dried extract)**
Opuntia elatior Mill crude extract	1	0.016	58

4.2.3 Discussion

To determine the total flavonoid content of the test samples the standard curve was used. For 1mg/ml concentration of crude extract of *Opuntia elatior* Mill crude extract 58 mg of AAE/gm of dried extract of flavonoid content was found. So it can be said that, the extract contains antioxidative compounds.

4.3 Result of Total Reducing Power Assay

The crude extract of *Opuntia elatior* Mill was subjected to determine total reducing power. Ascorbic acid was used as reference standard.

4.3.1 Preparation of Standard Curve

Table 4.5: Total reducing power of ascorbic acid

Concentration (µg/ml)	**Absorbance (At 700 nm)**	**Regression line**	**R^2 value**
250	2.657	y = 0.010x+0.266	0.821
200	2.126		
150	2.284		
100	1.603		
50	0.355		

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.

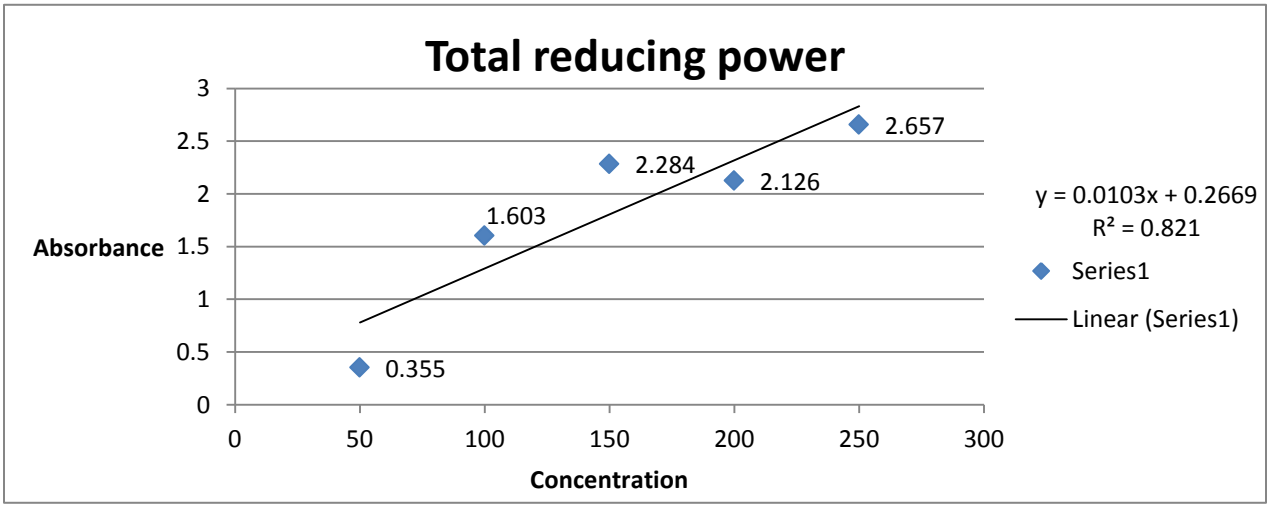


Figure 4.3 Graphical representation of assay of reducing power of ascorbic acid

4.3.2 Total Reducing Power Assay in crude extract of *Opuntia elatior* Mill

Based on the absorbance values of the extract solution, reacted with potassium ferricyanide reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total reducing power present in the crude extract is calculated and given in the table below.

Table 4.6: Total reducing power of *Opuntia elatior* Mill crude extract

Sample	**Concentration (mg/ml)**	**Absorbance (Y value)**	**Total reducing power (X) value (mg of AAE/gm of dried extract)**
Opuntia elatior Mill crude extract	1	1.337	111.1

4.2.1.3 Discussion

The absorbance was found to be directly proportional to the concentration in standard and crude extract samples. In both extract and standard the absorbance increased with the increase in concentration indicating increase in reducing power content. Compare with the absorbance of the extract is less than the standard.

Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 111.1 mg of AAE/gm of dried extract of reducing power content was found in the aqueous fraction of *Opuntia elatior* Mill crude extract.

4.4 Result of Brine Shrimp Lethality Bio-Assay

The *Opuntia elatior* Mill crude extract was subjected to brine shrimp lethality bioassay.

After 24hrs, the test tubes were inspected using a magnifying glass and the number of survivors counted.

The effectiveness of the concentration and % mortality relationship of plant product was expressed as a median Lethal Concentration (LC_{50}) value.

LC_{50} represents the concentration of the standard or 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.

4.4.1 Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard

Table 4.7 Results of the bioassay of Tamoxifen (standard)

Test tube number	**Concentration (µg /*ml*)**	**Log C**	**Number of alive nauplii**	**Number of dead nauplii**	**% Motality**	**Lethal concentration 50 or LC_{50} (µg /*ml*)**
1	400	2.602	0	10	100	25
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	3	7	70	
5	25	1.398	5	5	50	
6	12.5	1.097	5	5	50	
7	6.25	0.796	6	4	40	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	0.78125	-0.107	9	1	10	

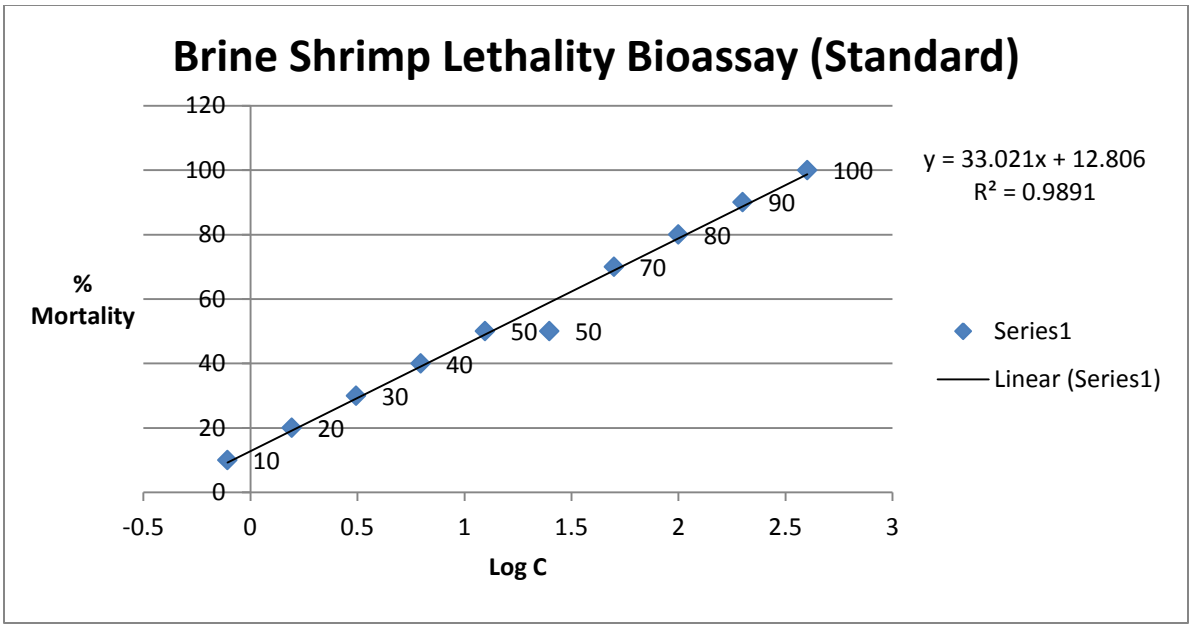


Figure 4.4: % mortality and predicted regression line of Tamoxifen (standard)

4.4.2 Preparation of crude extracts Curve of *Opuntia elatior* Mill:

Table 4.8 Results of the bioassay of crude extract.

Test tube no.	**Concentration (C) (µg/ml)**	**LogC**	**Number of nauplii alive**	**Number of nauplii dead**	**% Mortality**	**LC_{50} (µg/ml)**
1	400	2.602	1		90	
2	200	2.301	2	8	80	
3	100	2.000	2	8	80	
4	50	1.699	3	7	70	
5	25	1.398	4	6	60	
6	12.5	1.097	4	6	60	
7	6.25	0.796	5	5	50	6.25
8	3.125	0.495	6	4	40	
9	1.5625	0.194	8	2	20	
10	0.78125	-0.107	9	1	10	

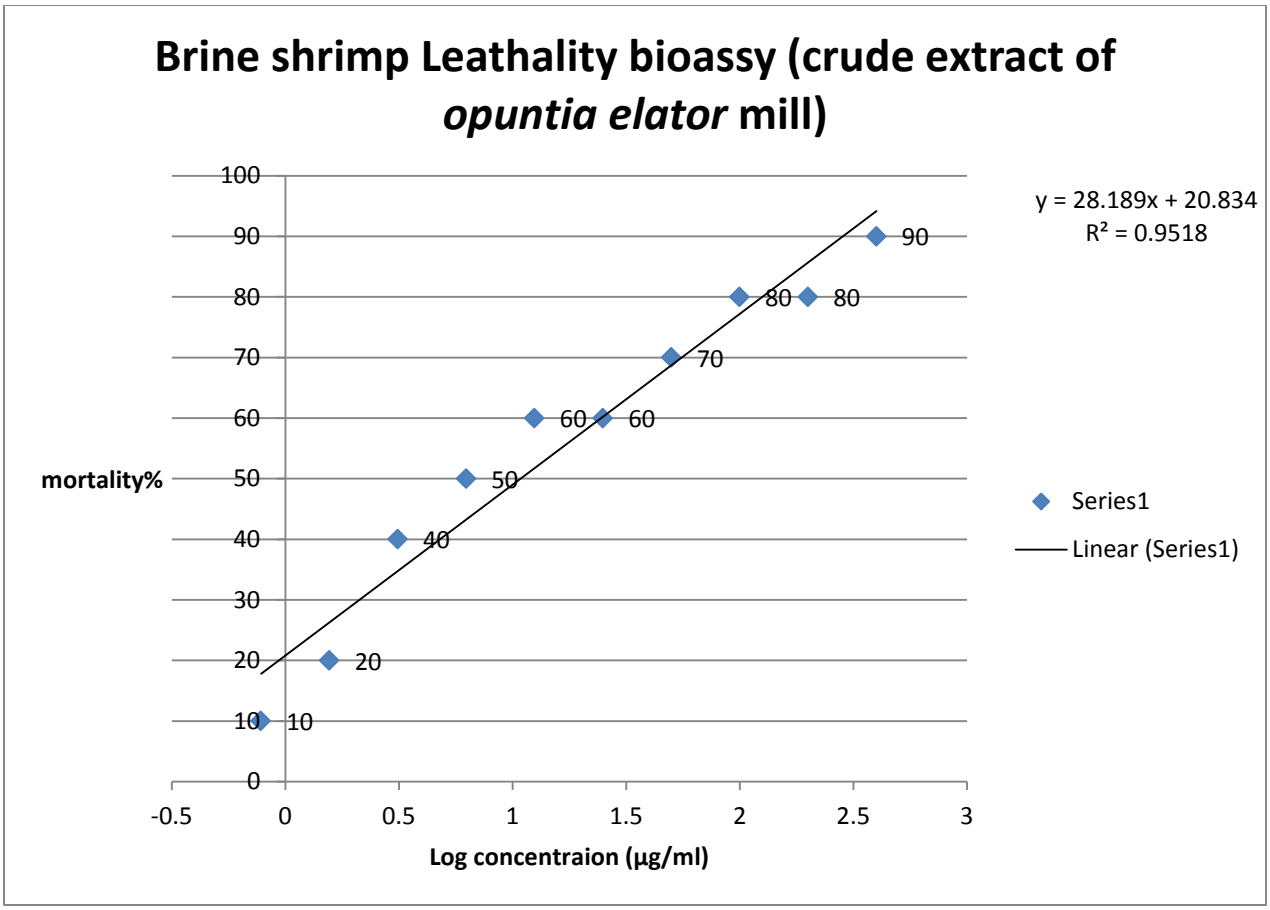


Figure 4.5:% mortality and predicted regression line of crude extract

4.4.3 Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional to the concentration ranging from the lowest concentration to the highest concentration in both standard and crude extract samples. Mortality increased gradually with an increase in concentration of the test samples. Maximum mortalities took place at the highest concentration of 400µg/ml, whereas the least mortalities at lowest concentration 0.78125µg/ml as shown in Table 4.7 and Table 4.8.

Table 4.9 Cytotoxic activity of Tamoxifen and crude extract of *Opuntia elatior* Mill

Sample	**Linear regression equation**	**R^2 value**	**LC_{50} (µg/ml, 24hr) (how to determine)**
Standard (Tamoxifen)	y = 33.021x + 12.806	0.989	25
Extract (Aqueous fraction)	y = 28.18x + 20.83	0.951	6.25

In this investigation, standard and crude extract exhibited cytotoxic activities with the LC_{50} values 25µg/ml and 6.25 µg/ml respectively as shown in Table 4.9.

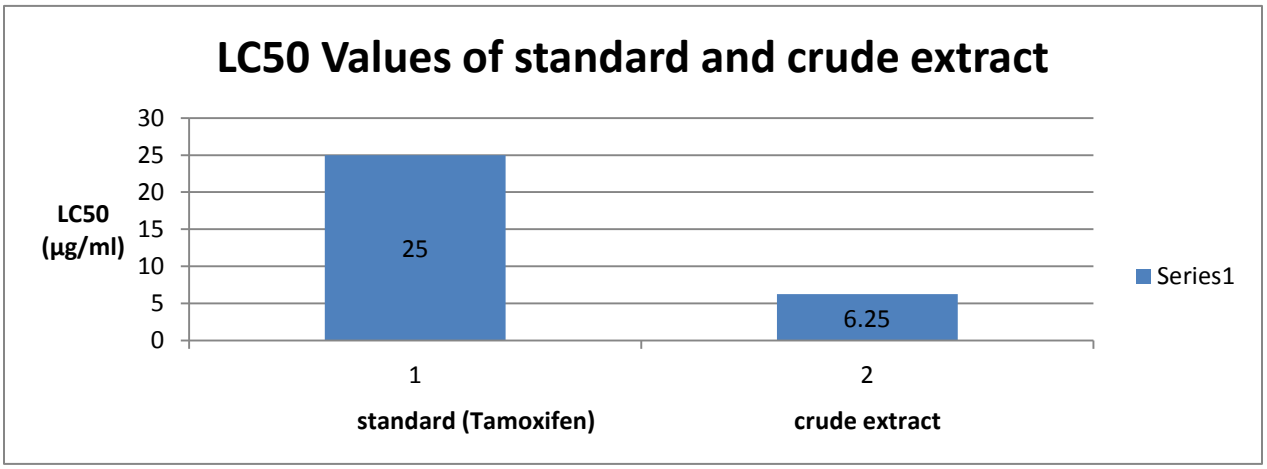


Figure 4.6: Comparison between LC_{50} values of standard and extract

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

Chapter: Five

CONCLUSION

5.1 Conclusion

Opuntia elatior Mill (Family: Cactaceae) commonly known as Phanimansa is an important species of *Opuntia* .The plant are more popular in traditional medicines to treat many disease.As the literature review suggests, the presence of several phytochemical compounds in crude extract of *Opuntia elator* Mill, makes the plant pharmacologically active.

The results obtained in this study indicate that the crude extract fraction of the plants of *Opuntia elator* Mill have significant cytotoxic activity. So the plants can be further evaluated for anticancer, pesticidal and antitumor properties.

Experimental evaluation showed that this plant also possess antioxidant properties. Investigations performed on the crude extract proved that the plant contain phenolic and flavonoid compounds. It has better reducing power .if any counteracting compounds present in a mixture.so pure compound isolation should be done in future to confirm the antioxidant properties of crude extract of *opuntia elator* Mill.

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

Chapter: Six

Reference

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