Cytotoxic and Antioxidant activity in aqueous fraction of *Opuntia elatior* extract

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

Submitted By

MD. Sahibul Alam

ID: 2011-1-70-037

Department of Pharmacy

East West University

Dedication

This Research Paper is dedicated to

My beloved parents, they are my biggest

inspirations.

DECLARATION BY THE CANDIDATE

I, MD. Sahibul Alam, hereby declare that this dissertation, entitled "Cytotoxic and Antioxidant activity of aqueous fraction of *Opuntia elatior* extract" submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

Md. Sahibul Alam

ID: 2011-1-70-037

Department of Pharmacy

East West University

Aftabnagar, Dhaka

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"**Cytotoxic and Antioxidant activity of aqueous fraction of *Opuntia elatior* extract" is a research work done, under our guidance and supervision by Md. Sahibul Alam (ID: 2011-1-70-037), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Abdullah-Al-Faysal

Lecturer

Department of Pharmacy,

East West University, Dhaka

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled "Cytotoxic and Antioxidant activity of aqueous fraction of *Opuntia elatior* extract is a bonafide research work done by Md. Sahibul Alam (ID: 2011-1-70-037), in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

Dr. Shamsun Nahar Khan

Chairperson

Associate Professor

Pharmacy Department

East West University

Aftabnagar, Dhaka

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ABSTRACT

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

The powdered of *Opuntia elatior* were extracted with methanol and then partitioned with pet-ether, DMSO, ethyl acetate and crude fraction was taken for experiment. The aqueous fraction was used to evaluate cytotoxic and antioxidant activities. The cytotoxic activity was measured by brine shrimp lethality bioassay. LC_{50} value of aqueous fraction of *Opuntia elatior* was 12.5µg/ml in brine shrimp lethality test. The fraction contained 12.315 mg AAE/g of total phenolic content, 30.5 mg AAE/g of total reducing power content and 56 mg AAE/g total flavonoid content. The results of study clearly indicate the presence of cytotoxic and poor antioxidant properties of aqueous extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

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

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Abbreviations:

Meaning of abbreviated form	Abbreviated form
Ascorbic Acid Equivalent	AAE
Dimethyl Sulfoxide	DMSO
Gram	g or gm
Hour	hr
Lethal concentration required to kill 50% of the sample population	LC_{50}
Microgram	µg
Micro liter	µl
Milligram	mg
Milliliter	ml
Opuntia	*O.*
Ultraviolet	UV
World Health Organization	WHO

CHAPTER ONE

INTRODUCTION

1.1 Plants:

Plants are the backbone of all life on Earth and an essential resource for human well-being.

- 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.
- Plants give us Oxygen as a byproduct of photosynthesis.
- One-quarter of all prescription drugs come directly from or are derivatives of plants. Additionally, four out of five people around the world today rely on plants for primary health care (Jarvis C, 1980).
- Plants use chlorophyll to capture light energy, manufacture food such as sugar, starch, and other carbohydrates.
- Plants regulate environment by storing carbon dioxide.
- There are over 260,000 species in the Plantae Kingdom (Greenman JM, 1915).

1.1.1 Medicinal Plants:

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs for the treatment of illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness. Typically pharmaceutical drugs are modeled after compounds found in medicinal plants (Study.com, 2015).

WHO (World Health Organization) defines “A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs” (Goldstein *et al.,* 1974).

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. Some plants possess some special qualities that make them medicinally important. Plants give some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal

properties. Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way medicinal plants play significant role of an economy of a country (Assignment Point, 2013).

1.1.2 The number of medicinal plants:

There are a huge number of medicinal plants. In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of the world's flowering plant species have been used medicinally (Catling PM and Small E).

1.1.3 Herb:

An herb can be any form of a plant or plant product, including leaves, stems, flowers, roots, and seeds.

Figure 1.1: Herbal plant

These plants can either be sold raw or as extracts, where the plant is macerated with water, alcohol, or other solvents to extract some of the chemicals. The resulting products contain dozens of chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, and

others. Because any given herb contains multiple ingredients, some manufacturers attempt to create standardized herbal products by identifying a suspected active ingredient and altering the manufacturing process to obtain a consistent amount of this chemical (Bent S, 2008).

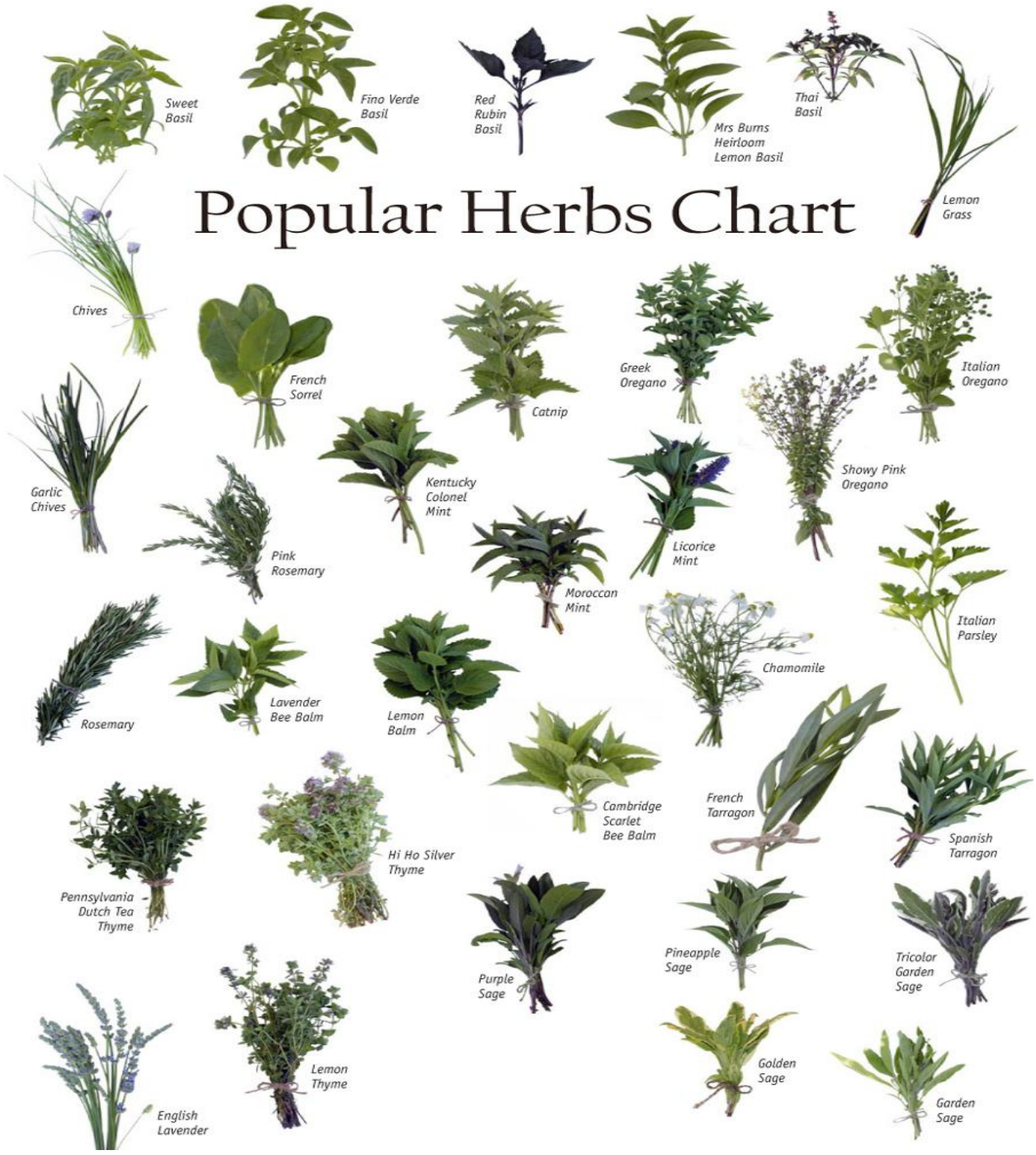


Figure 1.2: Important Herbs

1.1.4 Significances of Medicinal Plants to Human Being:

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin comes from willow bark.
- 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.
- 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 (Akhter S, 2004).

Figure 1.3: Willow bark

1.1.5 History of Plants in medicine:

- The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.
- The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Podophyllum*, ginseng, cinnamon bark, and ephedra.

- The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc.
- The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood was applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents.
- Theophrastus (371-287 BC) founded botanical science with his books "De Causis Plantarum"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore and monkshood. He describe about plant's toxic action.
- Dioscorides, "the father of pharmacognosy," wrote "De Materia Medica." 944 drugs became described in book, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect. Camomile, garlic, onion, marsh mallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion were described by Dioscorides.
- Galen (131 AD–200) had described *Uvae ursi folium* and a mild diuretic.
- Charles the Great (742 AD–814) had described sage, sea onion, iris, mint, common centaury, poppy, marsh mallow, etc.
- The Arabs used aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna and so forth. Certain drugs with strong action were replaced by drugs with mild action, for instance, *Sennae folium* was used as a mild laxative, compared to the purgatives *Hellebores odorous* and *Euphorbium* used until then.
- Paracelsus (1493-1541) was one of the proponents of chemically prepared drugs out of raw plants and mineral substances. For example, the haselwort is beneficial for liver diseases; St John's wort (*Hypericum perforatum* L.) would be beneficial for treatment of wounds and stings.

- The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy. With the upgrading of the chemical methods, other active substances from medicinal plants were also discovered such as tannins, saponosides, etheric oils, vitamins, hormones, etc.
- In 19th century, therapeutics, alkaloids, and glycosides isolated in pure form were increasingly supplanting the drugs from which they had been isolated. It was soon ascertained that although the action of pure alkaloids was faster, the action of alkaloid drugs was full and long-lasting.
- In early 20th century, stabilization methods for fresh medicinal plants were proposed, especially the ones with labile medicinal components. Besides, much effort was invested in study of the conditions of manufacturing and cultivation of medicinal plants (Petrovska B, 2012).

1.2 Why some of the plants are valued as medicinal plants:

Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism). Chemicals that make a plant valuable as medicinal plant are

- Alkaloids (compounds has addictive or pain killing or poisonous effect and sometimes help in important cures).
- Glycosides (use as heart stimulant or drastic purgative or better sexual health).
- Tannins (used for gastrointestinal problems like diarrhea, dysentery, ulcer and for wounds and skin diseases).
- Volatile/essential oils (enhance appetite and facilitate digestion or use as antiseptic and insect repellent properties).
- Fixed oils (present in seeds and fruits could diminish acidity).

- Gum-resins and mucilage (possess analgesic property that suppress inflammation and protect affected tissues against further injury and cause mild purgative).

- Vitamins and minerals (Fruits and vegetables are the sources of vitamins and minerals and these are used popularly in herbals) (Ghani A, 1998).

1.2.1 Natural Products in Medicine:

According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine.

- Mandrake was prescribed for pain relief
- Turmeric possesses blood clotting properties
- Roots of the endive plant were used for treatment of gall bladder disorders (Nahain *et al*., 2002).

- Paclitaxel from *Taxus brevifolia* used for the treatment of lung, ovarian and breast cancer.

- The alkaloid, forskolin from *Coleus forskohlii* and phytochemicals from *Stephania glabra*, are now being rediscovered as adenylate cyclase and nitric oxide activators, which may help in preventing conditions including obesity and atherosclerosis.

- Apomorphine is a semi synthetic compound derived from morphine (*Papaver somniferum*) used in Parkinson's disease.

- Cannabidiol obtained from cannabis plant (*Cannabis sativa*) and Capsaicin active compound from *Capsicum annuum* are used as pain relievers (Veeresham C, 2012).

1.2.2 The importance of medicinal plant in drug discovery:

Development of new drug is a complex, time-consuming, and expensive process. The time taken from discovery of a new drug to its reaching the clinic is approximately 12 years, involving more than 1 billion US$ of investments in today's context.

Essentially, the new drug discovery involves the identification of new chemical entities (NCEs), having the required characteristic of drug ability and medicinal chemistry. These NCEs can be

isolated from natural products. More than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources (including semi-synthetic analogs).

a) Morphine was isolated from opium produced from cut seed pods of the poppy plant (*Papaver somniferum*) approximately 200 years ago.
b) Few drugs developed from natural sources have undoubtedly revolutionized medicine, like

I. Antibiotics (e.g. penicillin, tetracycline, erythromycin), antiparasitics (e.g. avermectin).
II. Antimalarials (e.g. quinine, artemisinin), lipid control agents (e.g. lovastatin).
III. Immunosuppressant for organ transplants (e.g. cyclosporine, rapamycins).
IV. Anticancer drugs (e.g. paclitaxel, irinotecan).

Clinical trials are ongoing on more than 100 natural product derived drugs and at least 100 molecules/compounds are in preclinical development stage. Cancer and infections are the two predominant therapeutic areas for which the drug discovery program is based on natural products, but many other therapeutic areas also get covered, such as cardiovascular, gastrointestinal, inflammation etc.

The botanical sources are known to provide the following classes of NCEs for drug discovery processes.

- ✓ Bioactive compounds for direct use as drug, e.g. digoxin.
- ✓ Bioactive compounds with structures which themselves may act as lead compounds for more potent compounds, e.g. paclitaxel from *Taxus* species.
- ✓ The novel chemophore which may be converted into druggable compounds with/without chemical analoging.
- ✓ Pure photochemical for use as marker compounds for standardization of crude plant material or extract.
- ✓ Pure photochemical which can be used as pharmacological tools.
- ✓ Herbal extracts as botanical drugs, e.g. green tea extract (Katiyar *et al.*, 2012).

1.2.3 Use of Medicinal Plant in Bangladesh:

- The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country.
- A large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases.
- Traditional medicine practice in Bangladesh includes both the most primitive forms of folk medicine (based on cultural habits, superstitions, religious customs and spiritualism) as well as the highly modernized Unani and Ayurvedic systems (based on scientific knowledge and modern pharmaceutical methods and technology).
- More than four hundred big and small manufacturers in Bangladesh are now engaged in manufacturing traditional medicine preparations in various dosage forms using local and imported raw materials.
- Unani and Ayurvedic drugs manufactured in Bangladesh not only meet the local requirements but are also exported to the neighboring countries like Hamdard Laboratories Bangladesh. They improve their factories by installing modern equipment and machinery (Ghani A, 1998).

Table 1.1: List of the major medicinal plants of Bangladesh

Local name	**Scientific name**	**Figure**	**Medicinal use**
Ada	*Zinger officinale*		I. Stimulant II. Cough III. Tumor
Amloki	*Phyllanthus emblica*		I. Inflammation II. Ulcer III. Cancer

Arjun	*Terminalia arjuna*		I. Angina II. Diarrhea III. Urinary diseases
Ashoke	*Saraca indica*		I. Menstruation II. dysentery
Ban halud	*Curcuma aromatica*		I. Tonic II. Carminative
Biranga	*Embelia ribes*		I. Carminative II. Laxative III. Anthelmintic
Boch	*Acorus calamus*		I. Diarrhea II. Dysentery III. Cough IV. Tonic
Bohera	*Terminalia bellirica*		I. Cancer II. Cardiac diseases III. Stomach diseases
Dadmardan	*Cassia alata*		I. Anthelmintic II. Anti-parasitic

Datura	*Datura metel*		I. Hydrophobia II. Stimulant III. Cough IV. Mental disorder
Dhaifhul	*Woodfordia floribunda*		I. Stimulant II. Dysentery III. Diarrhea IV. Ulcer
Ghrita kumari	*Aloe indica*		I. Purgative II. Stomach diseases
Gobura	*Anisomeles indica*		I. Carminative II. Astringent III. Tonic
Golmarich	*Piper nigrum*		I. Cough II. Inflammation III. Carminative
Halud	*Curcuma longa*		I. Rheumatic fever II. Arthritis III. Lung and Skin cancer
Haritaki	*Terminalia chebula*		I. Ulcer II. Conjunctivitis III. Inflammation

Iswarmul	*Aristolochia indica*		I. Stimulant II. Tonic III. Stomach diseases IV. Anti- periodic
Kala halud	*Curcuma caesia*		I. Diarrhea II. Epilepsy III. Cancer
Kala megh	*Andrographi spaniculata*		I. Hepatitis II. Anthelmintic III. Dysentery IV. Fever
Lal Chitra	*Plumbago rosea*		I. Conjunctivitis, II. Skin diseases III. Leprosy IV. Syphilis
Lebugandhi ghas	*Cymbopogon citrates*		I. Stomach disease II. Rheumatic fever
Neem	*Azadirachta indica*		I. Tonic II. Antispasmodic III. Insecticide
Nishinda	*Vitex negundo*		I. Tonic II. Headache III. Vomiting IV. Malaria

Pipul	*Piper longum*		I. Cough II. Arthritis III. Asthma IV. Gonorrhea
Punarnova	*Boerhaavia diffusa*		I. Diuretic II. Antihelmenthic III. Skin diseases IV. Asthma
Sarpaganda	*Rauwolfia serpentine*		I. Hypertension II. Sedative
Shatamuli	*Asparagus racemosus*		I. Diuretic II. Diarrhea III. Tonic
Sheta Chandan	*Santalum album*		I. Bronchitis II. Skin diseases III. Fever
Tejpata	*Cinnamomum tamala*		I. Bronchitis II. Cough
Thankuni	*Centella asiatica*		I. Energetic (tonic) II. Skin diseases

Tulsi	*Ocimum sanctum*		I. Cough II. Dysentery III. Stomach diseases
Vasak	*Adhatoda vasica*		I. Expectorant II. Cough III. Asthma (Khan NA *et al.*, 2006).

1.2.3 Economic opportunities:

When a plant is popular medicinally, its commercial value is likely to lead to over collection. Many very important drug plants grow in the shade of trees (for examples, ginseng, goldenseal, and pacific yew) and, because they grow very slowly, are especially susceptible to over collecting. Such non-timber forest resources are of importance to the forest industry, which is looking for alternative crops. Cultivation offers the possibility of not only preserving economically important wild plants in their natural habitats, but also of providing farmers with new crops. Domestic and foreign markets for medicinal plants are growing rapidly and provide opportunities to build up a healthy economy for a country (Meares P, 1987).

1.3 Research on Herbal Drug:

Traditional herbal medicines are naturally occurring; plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates. In China, traditional herbal medicine played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS). Eighty per cent of African populations use some form of traditional herbal medicine, and the worldwide annual market for these products approaches US$ 60 billion. Many hope traditional herbal medicine research will play a critical role in global health. China, India, Nigeria, the United States of America (USA) and WHO have all made substantial research investments in traditional herbal

medicines. Industry has also invested millions of US dollars looking for promising medicinal herbs and novel chemical compounds. This is still a relatively modest investment compared to the overall pharmaceutical industry; however, it raises interesting ethical questions, some of which are not faced in more conventional drug development.

As attention and public funding for international traditional herbal medicine research collaborations grows, more detailed analysis of ethical issues in this research is warranted. Scant literature has addressed selected issues such as informed consent and independent review related to traditional herbal medicine research. Here we apply a practical, comprehensive and widely accepted ethical framework to international traditional herbal medicine research. We examine in detail difficult questions related to social value, scientific validity and favorable risk–benefit ratio. We conclude with implications for future research in this area, focusing on the importance of collaborative partnership (Tilburt J, 2008).

1.3.1 Combination of herbal drug:

Herbal drug may be defined as the plants, plant parts and plant products of all description, particularly those with medicinal properties. Herbal drugs are generally manufactured by the combination of two or more natural substances. The utility of these combinations are:

- To increase efficacy of the drug.
- To remove toxic effects. (combination needs small amount of plant product)
- To reduce side-effects. (Counteract each other)
- To maintain stability.
- To keep pleasant taste, color and odor.

1.3.2 Criticism of herbal drug:

Herbal products have gained increasing popularity in the last decade, and are now used by approximately 20% of the population. Herbal products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant, including leaves, stems, flowers, roots, and seeds. Under the current law, herbs are defined as dietary supplements, and

manufacturers can therefore produce, sell, and market herbs without first demonstrating safety and efficacy, as is required for pharmaceutical drugs. Although herbs are often perceived as "natural" and therefore safe, many different side effects have been reported owing to active ingredients, contaminants, or interactions with drugs.

Of the top 10 herbs, 5 (ginkgo, garlic, St. John's wort, soy, and kava) have scientific evidence suggesting efficacy, but concerns over safety to use these products.

Herbal products are not likely to become an important alternative to standard medical therapies unless there are changes to the regulation, standardization, and funding for research of these products (Bent S, 2008).

1.4 Botanical medicine used in common health conditions:

Cardiovascular and circulatory functions:

Clinical studies indicate that botanical medicines have applications both for the maintenance of cardiovascular and circulatory health, and also for treatment of cardiovascular dysfunctions such as arrhythmia and mild hypertension (or high blood pressure). For example, numerous clinical and animal studies document the efficacy of hawthorn as a cardio tonic. Cardio tonics help to improve blood supply to the heart, increase the tone of the heart muscle, stimulate cardiac output, dilate coronary arteries, stabilize blood pressure, prevent atherosclerosis (the accumulation of arterial plaque), and prevent or help improve congestive heart failure. Many herbs used for cardiovascular health, such as hawthorn and gingko, have antioxidant properties, which may help prevent hardening of the arteries or other circulatory insufficiencies.

Figure 1.4: Hawthorn and Garlic

Some herbs used for cardiovascular health are commonly taken to lower cholesterol. Garlic is one notable example, and a number of clinical studies have shown that garlic is effective in moderately reducing serum cholesterol (Frishman WH *et al.*, 2005).

Digestive, gastrointestinal, and liver functions:

Clinical research indicates that ginger is a very effective herb for nausea, indigestion, and minor gastric upsets. Ginger is also effective for morning sickness in the early stages of pregnancy and for motion sickness. Peppermint oil has demonstrated clinical efficacy for irritable bowel syndrome. Many herbs are liver protective and restorative-they can help to protect a healthy liver and restore function to a liver that has suffered impaired functions due to disease or injury, such as cirrhosis, hepatitis, or exposure to hepatotoxic agents. The potential benefit of milk thistle in the treatment of liver diseases (Langmead L and Rampton DS, 2001).

Figure 1.5: Peppermint, Milk thistle and Ginger

Endocrine and hormonal functions:

Adaptogenic herbs, such as ginseng, owe much of their activity to stimulation of pituitary and adrenal activity (Gaffney BT *et al.*, 2001).

Figure 1.6: Ginseng

Renal functions:

Many plants are diuretics. They can help eliminate disease-carrying microorganisms from the urinary tract, and they can help prevent kidney stone formation and bladder inflammation resulting from bladder irritation-whether or not it's due to microbial infection. Others are effective urinary tract disinfectants. One that has been studied clinically and found effective for both prevention and treatment of urinary tract infections is cranberry, which may be taken as cranberry juice or in the form of concentrated cranberry juice solids (Jepson RG and Craig JC, 2007).

Figure 1.7: Cranberry

Reproductive functions:

Helping milk production: Some nursing women use herbs to induce milk production during lactation, or conversely, to reduce milk production during weaning. For example, Fenugreek is an herb that has been successfully used to induce lactation.

Treating menopausal: Black cohosh and red clover are effective for treating menopausal symptoms (Gabay MP, 2002).

Figure 1.8: Red clover

Inflammations, and cancer:

Reducing inflammation: Anti-inflammatory botanicals, of which there are many (examples include ginkgo, ginger, hawthorn, and St. John's wort) are useful in suppressing various immune functions involved in the inflammatory response (Geller SE and Studee L, 2005).

Prevention or treatment of cancer: Cancer is basically caused by a failure of the immune system to recognize and destroy cancerous cells. Extracts of immune-stimulating medicinal mushrooms, such as reishi or turkey tail can be used as adjunct therapies to help maintain immune functions during radiation and chemotherapy (Park EJ and Pezzuto JM, 2002).

Nutritional functions:

Antioxidants: Fruit and vegetables rich in antioxidants are best as antioxidant supplements.

- Flavonoid-containing herbs, such as ginkgo, hawthorn, and grape seed extracts
- Green tea, in its natural form or as a concentrated supplement
- Dark chocolate contains many of the same beneficial compounds, known as catechins (Engler M and Chen C, 2004).

Diabetes and Carbohydrate Metabolism:

One application of botanical medicines in this area is to lower blood sugar in individuals who may be diabetic or pre-diabetic. Popular botanical medicines thought to have this effect include:

- Ginseng
- Ayurvedic medicine
- Green tea (Chantre P and Lairon D, 2002).

Figure 1.9: Green tea

1.5 Necessity of herbal drug research in Bangladesh:

Most of the people of our country have little access to allopathic medicine due to their low income in respect of high cost of allopathic medicine.

A survey conducted in 1990 in different villages of Bangladesh shows that:

- 18% people contact qualified allopathic doctors,
- 37% contact unqualified village doctors,
- 18% contact mollahs,
- 27% contact homeopaths.

The survey indicates an extensive use of medicinal plants, most of which are served in a crude and substandard form, by our people. The use of such crude and substandard herbal drug is dangerous and may threaten public health. Thus the analysis of plants for exploring the bounty of chemical entities and their biological screening is the current need for standardization of herbal medication. A proper health care system can be supplied to population by utilizing our natural resources of medicinal plants and their constituents. Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. During the last five years Bangladesh has spent more than 1500 crore Taka for importing chemicals, raw materials and semi-processed drugs of plant origin from neighboring and other countries and this trend is growing upwards day by day. This huge foreign exchange can be saved if the indigenous medicinal plants or its semi processed products are utilized by the manufacturer to satisfy their need.

1.6 Natural Sources: A Model for Synthetic Drugs:

Natural sources are contributing to the development of modern synthetic drugs:

i. Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.

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

iii. Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs (Ghani A, 1998).

1.6.1 Necessity of Drug Development from Plant Sources:

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role. Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents.

I. Vincristine, a prominent anticancer drug, was developed from periwinkle plant (*Vincarosea*) which was formerly prescribed for treating diabetes. The efficient hypotensive drug, reserpine, was developed from *Rauwolfia serpentine* which was previously provided as an antidote.

II. Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammivi snaga* which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Chopra RN *et al*., 1982).

1.6.2 Procedure for Development:

Since drug development is an expensive practice, careful photochemical analysis and pharmacological screening and if promising clinical tests are required.

The way of developing drugs from plants involves several stages which include:

I. Selection and correct identification of the proper medicinal plant.

II. Extraction with suitable solvents.

III. Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.

IV. Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.

V. Repeated fractionation of active fractions to isolate pure compounds.

VI. Elucidation of chemical structure of pure compounds using spectroscopic methods.

VII. Evaluation of biological activity of pure compounds.

VIII. Toxicological tests with pure compounds.

IX. Production of drug in appropriate dosage forms (Ghani A, 1998).

1.6.3 Bioactivity Guided Research of Medicinal Plants:

Success in natural products research is conditioned by a careful plant selection, based on chemotaxonomic data, information from traditional medicine, field observations. One main strategy in the isolation of new leads consists of the so-called Bioactivity-guided isolation, in which pharmacological assays are used to target the isolation of bioactive compounds.

Bioactivity guided phytochemical approach, has three phases of investigation.

- ✓ Firstly, biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.
- ✓ Secondly, the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained.
- ✓ Thirdly chemical structures of pure compounds are determined (Grabley and Thiericke, 1999).

1.7 Name and Distribution of *Opuntia elatior*:

Scientific name: *Opuntia elatior*.

Common names: Prickly Pear, Slipper Thorn, Chau, Shangran, Tuna, Nag Phani, Nagatali.

Bengali names: Phanimansa, Naghana, Nagphana.

Distribution: It can tolerate a wide range of temperature and moisture levels, it grows best in sunny desert-like conditions. It grows best in North and South America, India, Bangladesh, Indonesia, Australia, West Indies, Mexico and South Africa (Mpbd.info).

1.7.1 Taxonomic classification:

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Streptophyta
Superdivision:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Superorder:	Caryophyllanae
Order:	Caryophyllales
Family:	Cactaceae
Subfamily:	Opuntioideae
Genus:	*Opuntia*
Species:	*Opuntia elatior* (ITIS.gov).

1.7.2 Description of *Opuntia elatior*:

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. Fruit is egg shaped as with most *Opuntia*s, and reddish to reddish-pink when ripe; the outer surface of the fruit is typically tuberculate, as a result of the raised areoles; small spines may develop from the areoles of the fruit. Spines are clustered in groups of two to eight, each 2-4 cm long, needle-like and bright white aging darker grey to brown. 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, the style is expanded at the base forming nectar, the filaments are reddish-pink, and the stigma lobes are green. Berry pyriform, truncate, depressed at the apex (Mpbd.info).

Figure 1.10: Flowers of *Opuntia elatior*

Figure 1.11: Leaves of *Opuntia elatior*

Figure 1.12: Fruits of *Opuntia elatior*

1.8 Uses of *Opuntia elatior*:

Immune System Booster: A single serving of *Opuntia elatior* contains more than 1/3 of entire daily requirement of vitamin C, also known as ascorbic acid. Vitamin C plays a major role in the immune system, stimulating the production of white blood cells and acting as an antioxidant throughout the body. Furthermore, vitamin C is an important component of various enzymatic and metabolic processes, including the creation of bone and muscle tissue.

Builds Strong Bones and Teeth: Calcium is an integral part of the human diet, and *Opuntia elatior* contain a significant level of calcium in every serving. Calcium is a very important element in the creation of bone tissue in the body; in fact, 99% of the calcium in body is found in bones and teeth. By ensuring enough calcium, age-related bone disorders like osteoporosis can be prevented.

Digestive Health: *Opuntia elatior* have a significant level of dietary fiber, like most fruits and vegetables, so these spiny fruits can help to regulate digestive process. Fiber bulks up stool to help food pass through the digestive tract easier, thereby eliminating constipation, bloating, and more serious gastrointestinal issues, such as colon cancer or gastric ulcers.

Heart Health: There are a number of components of *Opuntia elatior* that make it very good for heart health. First of all, the levels of fiber in the fruit can help to lower the levels of "bad" cholesterol in the body, while the significant levels of potassium can help to lower blood pressure, by relaxing the blood vessels and reducing the stress on the cardiovascular system. Finally, the betalains found in prickly pear, have been directly connected to strengthening the endothelial walls of blood vessels, thereby reducing the chances of weakening or damage to the system. Reducing cholesterol, lowering blood pressure, and strengthening blood vessels can prevent atherosclerosis, coronary heart disease, and strokes.

Cancer Prevention: *Opuntia elatior* have high levels of flavonoids, polyphenols, and betalains, all of which act as antioxidant compounds and neutralize free radicals before they can cause healthy cells to mutate into cancerous cells.

Antioxidant Potential: This antioxidant protect skin health, lower the chances of premature aging, improve vision, prevent macular degeneration, and also increase the strength and functionality of brain. Free radicals are partially responsible for the oxidation of neural cells that can lead to dementia and Alzheimer's disease. Polyphenolic compounds have been linked to increased cognitive activity.

Weight Loss Efforts: Like all fruits with high fiber and impressive nutrient density, combined with very low calories and very low saturated fat, *Opuntia elatior* can keep body in healthy form without packing on any extra weight and the fiber and carbohydrates prevent from overeating.

Diabetes: Single doses of *Opuntia elatior* can decrease blood sugar levels by 17% to 46% in some people. *Opuntia elatior* contains fiber and pectin, which can lower blood glucose by decreasing the absorption of sugar in the stomach and intestine.

Hangover: Taking *Opuntia elatior* before drinking alcohol might reduce some symptoms of hangover the next day. It seems to significantly reduce nausea, anorexia, and dry mouth. But it does not seem to reduce other hangover symptoms such as headache, dizziness, diarrhea, or soreness.

Enlarged prostate: Men with an enlarged prostate often feel their bladder is full, and they experience frequent, strong urges to urinate. Developing evidence suggests that taking powdered *Opuntia elatior* flowers may reduce these symptoms.

High blood cholesterol: Early research shows that taking edible pulp of *Opuntia elatior* daily, while following a diet, can reduce total cholesterol, low-density lipoprotein (LDL or "bad") cholesterol, and triglyceride levels in people with high cholesterol. High-density lipoprotein (HDL or "good") cholesterol levels do not seem to be affected (Organicfacts.net).

Healing purposes: *Opuntia* has been used for healing purposes, cuts and bruises**,** sunburn, windburn, constipation and cold symptoms. Folk remedies abound, such as the one that involves heating the pads and placing them on a cold sufferer's chest to relieve congestion (Park EH and Chun MJ, 2001).

Inflammation: In traditional medicine, *Opuntia elatior* was mashed and applied topically to parts of the body that were inflamed. When consumed, some of the antioxidant and mineral contents of *Opuntia elatior* can also lower inflammation, particularly with conditions like arthritis, gout, or muscle strain. It can also be topically applied to eliminate the swelling of bug bites, a method that has been in use for hundreds of years (Loro J *et al.*, 1999).

1.8.1 Use of various parts of *Opuntia elatior*:

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, asthma and gonorrhea (Mpbd.info).

1.8.2 Medicinal drug of *Opuntia elatior*:

Opuntiol: Opuntiol (2-Hydroxymethyl-4-methoxy-2*H*-pyran-2-one) drug derived from fruit of *Opuntia elatior* inhibits acid corrosion.

The anticorrosion ability of *Opuntia elatior* fruit extract was tested on mild steel (MS) in 1 M HCl and H2SO4 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. 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 *Opuntia elatior* can be related to the presence of opuntiol (Loganayagi C *et al*., 2014).

1.8.3 Nutritional importance:

Cacti have long been considered an important nutritional source (bread of the poor) in Latin America, among which *Opuntia* has gained highest economic importance worldwide. It is cultivated in several countries such as Mexico, Argentina, Brazil, Tunisia, Italy, Israel and China. Both fruit and stems have been regarded to be safe for consumption as food. The constantly increasing demand for nutraceuticals is paralleled by a more pronounced request for natural ingredients and health-promoting foods. The multiple functional properties of cactus pear fit well in this trend. Recent data reveal the high content of some chemical constituents, which can give added value to this fruit on a nutritional and technological functionality basis. High levels of betalains, taurine, calcium, magnesium, and antioxidants are noteworthy (Feugang *et al*., 2006).

1.9 Aims of the Present Study:

Attempts should be continued for the evaluation of the cytotoxic, antimicrobial, and antioxidant activity of the aqueous fraction of *Opuntia elatior* extract. To conduct cytotoxic investigation of aqueous extract by brine shrimp lethality bioassay. To investigate in vitro antioxidant property of aqueous extract by DPPH free radical scavenging assay, total Phenolic content and total Flavonoid content. *Opuntia elatior* is a very common plant which is used in our country as well as in world by a lot of people for several purposes. All the parts of this plant are used for medicinal activity. To achieve this objective, the whole work was designed in the following way:

I. Cytotoxic study with aqueous fraction.

II. Observation of antimicrobial action with aqueous fraction.

III. Antioxidant study with aqueous fraction (Chahdoura *et al*., 2014).

CHAPTER TWO

LITERATURE REVIEW

2.1 Phytochemical Studies of *Opuntia elatior*:

Phytopharmaceutical products have served as a major source of drugs for centuries and today about half of the pharmaceuticals in use are derived from natural products (Thirupati K *et al*., 2008).

The presence of potentially active nutrients and their multifunctional properties make *Opuntia spp.* fruits and cladodes perfect candidates for the production of phytopharmaceutical products (Feugang *et al*., 2006).

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 (Mpbd.info).

Ripe fruits of *Opuntia elatior* Mill. Contain red pigment i.e. betanin which is betacyanin pigment. Betacyanin was confirmed by spectrophotometric and high performance liquid chromatography coupled with mass spectroscopy techniques. The average weight of fruit was 24.568 ± 7.134 g/unit and the percentage of peel and seed was very low compared to the edible portion. Phytochemical analysis indicated the presence of color pigment betacyanin as an active principle and sugar content in high amount and low acidity of fruit which make it very sweet and delicious. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher as compared to *Opuntia ficus-indica* and *Opuntia undulata Griff*. while lower as compared to *Opuntia stricta Haw* (Chauhan SP *et al*., 2013).

2.2 Antioxidant property of *Opuntia elatior*:

Total phenolic, flavonoid, flavonone and betanin content of *Opuntia elatior* extract were found to be 52.76 mg/g, 39.22 mg/g, 9.60 mg/g and 47.10 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 P *et al.*, 2012).

The experimental data indicated that the Hydro-alcoholic extract (HAOE), ethyl acetate (EAOE) and butanol (BFOE) soluble fractions of *Opuntia elatior* Mill. have shown significant antioxidant activity. The highest Polyphenolic, Flavonoids (FA), Flavanone (FO) contents and degree of polymerization were found in EAOE.

The scavenging potential was in the order of Ascorbic Acid > EAOE > BFOE > HAOE > BIOE (n-butanol insoluble fraction), 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 P *et al*., 2014).

2.3 Analgesic action of *Opuntia elatior* fruits:

Fruit juice of *O. elatior* Mill. exhibited central and peripheral analgesic properties since it exerted a significant and dose-dependent protective effect on chemical (acetic acid injection) and thermal painful stimuli. Such an effect on the two stimuli is characteristic of central analgesics such as morphine and tramadol, while peripheral analgesics such as diclofenac sodium and aspirin are known to be inactive on thermal painful stimuli.

Fruits of *O. elatior* Mill. attenuated the nociceptive responses to chemical stimuli in the acetic acid-induced abdominal constriction. The mean number of abdominal contractions was reduced from 25 to 7 at the respective doses of 5 and 15 ml/kg. Diclofenac sodium, the peripheral analgesic drug, also produced a similar antinociceptive action. Acetic acid acts indirectly by inducing the release of endogenous mediators.

Fruit juice of *O. elatior* Mill. contains morphine- and tramadol-like components and other peripherally acting principles. Fruits of *O. elatior* Mill. is endowed with central and peripheral analgesic properties for the presence of phenolics and betanin content.

2.4 Anti-inflammatory action of *Opuntia elatior* fruits:

Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators.

Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs and has been frequently used to assess the anti-edematous effect of natural products.

Edema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) due to the release of kinin-like substances, especially bradykinin. The second phase is sensitive to most clinically effective anti-inflammatory drugs.

The anti-edematogenic mechanism of the action of *O. elatior* Mill. fruit is related to prostaglandin synthesis inhibition. Inflammation pain results from the release of hyperalgesic mediators - prostaglandins and catecholamines - which are supposed to act by regulating the sensitivity of pain receptors.

Neutrophils are present in much larger numbers than any other inflammatory cell in the circulation and in tissue stores, particularly the lung. Neutrophils are one of the first inflammatory cells to be recruited into the airways after either allergen exposure or injury.

Neutrophil adhesion to endothelium is enhanced by the activation of adenosine A_1 receptors. Binding of neutrophils to the adenosine A_2 receptor results in the inhibition of the respiratory burst reaction and decreased binding to fibrinogen.

Fruit juice of *O. elatior* Mill. significantly reduced the percentage of neutrophil adhesion. This may help in decreasing the release of various cytokines and might be able to bind to A_1 and/or A_2 receptor on the endothelium (Chauhan SP *et al*., 2015).

2.5 Mast cell degranulation property of *Opuntia elatior* fruits:

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine,

leukotrienes (LTs), platelet activating factors and chemotactic factors for eosinophils, neutrophils etc., from mast cells.

Fruit juice of *O. elatior* Mill. was found to inhibit the degranulation of mast cells induced by an immunological and a nonimmunological stimulus. Fruit juice of *O. elatior* Mill. able to interfere the release and synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Cytoprotective effect induced by fruit juice of *O. elatior* Mill. on mast cell surface could be due to its ability to alter the influx of calcium ions.

The IC50 value of *O. elatior* Mill. was found 12.24 and 18 µl/ml for immunological and nonimmunological induced mast cell degranulation. The betacyanin is an active principle compound in prickly pear that may responsible for mast cell stabilizing action (Chauhan SP *et al*., 2015).

2.6 Antidiabetic property of *Opuntia elatior*:

The hypoglycemic effects of *Opuntia* species are documented in numerous studies. One open-label study of 14 patients found that *O. streptacantha* decreased glucose and insulin levels in patients with noninsulin-dependent diabetes mellitus; however, the plant had no effect on glucose or insulin levels in healthy volunteers. Another open-label study involving 32 patients with type 2 diabetes treated with *O. streptacantha* also resulted in decreased glucose and insulin levels (Alarcon AFJ *et al*., 2003).

Opuntia was widely known for its use in herbal medicines to treat many diseases. Polysaccharides of *Opuntia monacantha* cladode (POMC) were extracted by distilled water and classified by ethanol solution with different concentrations. POMC could decrease the daily water consumption in streptozotocin-induced diabetic rats comparable to dimethylbiguanide, a commercial anti-diabetic drug. An increase to food intake was also shown for streptozotocin-induced diabetic rats administered by POMC. By determination of blood glucose (BG), total cholesterol (TC), total triglyceride (TG) and high density lipoprotein cholesterol (HDL) levels, it revealed that POMC had beneficial effects on the improvement in the control of blood glucose and serum lipid level. Daily treatment with 100–300 mg/kg body weight of POMC for four weeks not only brought a significant decrease on blood glucose level in streptozotocin-induced

diabetic rats, but also enhanced the cardioprotective lipid HDL level ($P < 0.05$). The insulin level in streptozotocin-induced diabetic rats was not significantly affected by POMC and dimethyl biguanide treatment ($P > 0.05$). The mechanism of POMC's hypoglycemic action might be similar to that of dimethyl biguanide.

Polysaccharides in it might be responsible for these beneficial properties. Polysaccharides were prepared from *Opuntia cladode* and their hypoglycemic effects were determined. The results indicated that this polysaccharides could improve the control in blood glucose and serum lipid levels of streptozotocin-induced diabetic rats. This conclusion would be helpful for further developing this traditional medicinal herb (Yang N *et al.*, 2008).

2.7 Antihyperlipidemic property of *Opuntia elatior*:

In one small study of 29 patients, prickly pear significantly reduced cholesterol levels. Prickly pear has antiplatelet activity, which is useful in patients with prothrombotic conditions such as diabetes and hyperlipidemia. Eight healthy volunteers and 8 patients with familial heterozygous hypercholesterolemia were treated with prickly pear 250 mg for 2 months. Significant ($P > 0.01$) decreases in total and LDL-cholesterol and reduced platelet proteins were found (Frati MA *et al.*, 1983).

The purpose of this study was to evaluate the hypolipidemic effect of a methanolic extract from *Opuntia joconostle* seeds fed to mice in a hypercholesterolemic diet. Acute toxicity of the methanolic extract was investigated by an established method. Phenolic composition and antioxidant activity were determined by high-performance liquid chromatography and DPPH, respectively. The total phenolic content of *Opuntia joconostle* seeds was 47.85 ± 1.29 mg gallic acid equivalents/g dry weight. The main phenolic compounds were identified as quercetin, rutin, and cafeic acid. Percent inhibition of $DPPH^+$ was 49.76 ± 0.49 %. The oral LD_{50} for the methanolic extract from the *Opuntia joconostle* seeds was >5000 mg/kg BW. Mice fed a hypercholesterolemic diet for six days exhibited significantly ($P \leq 0.001$) higher plasma lipid levels than mice fed a normal diet. Remarkably, supplementation with methanolic extract from *Opuntia joconostle* at doses of 1, 2, and 5 g/kg body weight significantly ($P \leq 0.001$) prevented the increase in total cholesterol, low-density lipoprotein cholesterol, triglycerides level, and atherogenic index. Similar concentrations of the HDL cholesterol were observed in both treated

and control groups. A significant dose-dependent reduction in lipid levels was noted for treated groups compared to the hypercholesterolemic group. We attribute this result to the seeds' phenolic composition. This methanolic extract has potential to be included in short-term hypercholesterolemia treatment regimens as it exhibits hypolipidemic activity with no apparent toxic manifestations (Osorio EO *et al*., 2012).

2.8 Antiulcer activity of *Opuntia elatior*:

A cytoprotective mechanism is associated with an interaction between the mucilage monosaccharides from prickly pear and membrane phospholipids. Cladodes of prickly pear is effective against the formation of ethanol-induced ulcer (Galati EM *et al.*, 2007).

Alcoholic extract of *Opuntia elatior* at 100, 200 and 400 mg/kg doses significantly ($P < 0.01$) reduced the ulcer score, ulcer number, ulcer index, 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 (Sivasubramanian P *et al.*, 2013).

2.9 Diuretic property of *Opuntia elatior*:

Natriuresis, kaliuresis and fructose-induced hyperuricemia reduce by *Opuntia ficus-indica.* Cladode, Fruit and Flower infusions of *Opuntia ficus-indica* significantly increase diuresis. This effect is more marked with the fruit infusion and it is particularly significant during the chronic treatment. The fruit infusion shows also antiuric effect. Cladode, flower and fruit infusions showed a modest but not significant increase in natriuresis and kaliuresis (Galati EM *et al*., 2007).

2.10 Wound healing property of *Opuntia elatior*:

Polysaccharide extracts from prickly pear cladodes enhanced cutaneous repair and healing of large, full-thickness wounds in a rat model. The polysaccharides from prickly pear cladodes have chondroprotective effects in treating joint diseases (Park EH and Chun MJ, 2001).

The wound healing activity of the mucilaginous and methanol extracts of *O. ficus-indica* flowers were assessed using excision wound model in rats. After thirteen days of treatment by both extracts, a beneficial effect on cutaneous repair was observed as assessed by the acceleration of wound contraction and remodeling phases. Histopathological studies of the granulation tissue indicated that the derma is properly arranged with the *Opuntia* flowers extract, compared with the control group. The mucilage extract was more effective than the methanol extract, but both showed significant results compared with the control. Such investigation was supported by the efficiency of the methanolic and mucilage extract as antimicrobial and antioxidant. Indeed, the extracts showed a potential antioxidant activity determined by different test systems, namely DPPH radicals scavenging activity, trolox equivalent antioxidant capacity, reducing power, β-carotene bleaching assay and metal chelating activity and exhibited significant antibacterial activity against almost all tested bacteria (Ammar I *et al.*, 2015).

2.11 Immunomodulatory and Antiasthmatic action of *Opuntia elatior*:

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. 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. 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 (Chauhan SP *et al.*, 2015).

2.12 Reversible antifertility potential of *Opuntia elatior*:

To examine the contraceptive efficacy of *Opuntia elatior* fruits on male mice two treatment groups of 250 and 500 mg/ Kg bw, was selected along with control. The final body weight, weight of testis and accessory reproductive organs of mice along with total sperm count and sperm abnormalities were recorded after treatment. A significant reduction in the weight of the

testis and epididymis was noticed in 500 mg/ Kg bw compared to control. In 500 mg/ Kg bw treated animals an 18.12% reduction in the sperm count and 17.82% increase in the total percentage of abnormal spermatozoa was found to be significant count. The fertility indices with 36.08% decrease in the litter size indicate the contraceptive effect of the fruit extract on mice. The weight of the testis and epididymis, total sperm count and the percentage of the abnormal spermatozoa were returning to the normal levels after the cessation of the treatment for 30 days. Thus the extract from *O. elatior* fruit shall be an effective contraceptive agent to regulate male fertility.

Withdrawal of the treatment for two weeks led to recovery of the epididymal sperm count, testicular HSDH activity, serum testosterone levels and the fertility. The methanol extract of the fruit of *O. elatior* shows reversible male antifertility activity without affecting the serum testosterone levels and libido (Ramya MC *et al.*, 2015).

2.13 Improved platelet function by *Opuntia elatior*:

Prickly pear is traditionally used by Pima Indians as a dietary nutrient against diabetes mellitus. Wolfram examined the effect of daily consumption of 250 g in eight healthy volunteers and eight patients with mild familial heterozygous hypercholesterolemia on various parameters of platelet function. Beside its action on lipids and lipoproteins, prickly pear consumption significantly reduced the platelet proteins (platelet factor 4 and β-thromboglobulin), ADP-induced platelet aggregation and improved the platelet sensitivity (against PGI2 and PGE1) in volunteers as well as in patients. Also, plasma 11-DH-TXB2 and the WU-test showed a significant improvement in both patients and volunteers. In contrast, collagen-induced platelet aggregation and the number of circulating endothelial cells showed a significant response in the patients only. Prickly pear may induce at least part of its beneficial actions on the cardiovascular system by decreasing the platelet activity and thereby improving the haemostatic balance (Wolfram *et al*., 2003).

2.14 Antiviral activity of *Opuntia elatior*:

Administration of cactus stem extract (*O. streptacantha*) to mice, horses, and humans inhibits intracellular replication of a number of DNA- and RNA-viruses such as Herpes simplex virus Type 2, Equineherpesvirus, pseudorabiesvirus, influenza virus, respiratory syncitial disease virus

and HIV-1. [47] Inactivation of extra-cellular viruses was also reported by the same authors. However, the active inhibitory component(s) of the cactus extract used in this study was not investigated, and as of yet, no further study has dealt with this specific topic. The efficacy of the crude extract of *O. vulgaris* against Newcastle virus disease in domestic fowl in Tanzania was evaluated (Mtambo MM *et al*., 1999).

2.15 Monoamino-oxidase inhibition property of *Opuntia elatior*:

Besides catechol methyl transferases, the monoamino-oxidases (MAOs) are usually involved in the catabolism of catecholamines, thus regulating the overall amine pool. In cladodes and fruits from the Korean *O. ficus-indica*, methyl esters derived from organic acids were identified as MAO inhibitors. The aqueous extracts showed least inhibitory activity, followed by the n-butanol fraction and the hexane extract, whereas the ethyl acetate fraction exerted the highest inhibitory action. The active agents were identified as 1-methyl malate, 1-monomethyl citrate,1,3-dimethylcitrate,and1,2,3-trimethylcitrate.Thepurified components showed MAO-A inhibitory action with increasing number of methyl substituents, whilst superior MAO-B inhibitory action was shown by 1-methylmalate compared to the mono- and dimethylcitrates. However, 1,2,3-trimethylcitrate exerted the strongest inhibition on both MAOs. When citrate was compared with its corresponding methyl derivatives, the methoxy moiety proved to be the effective moiety (Han YN *et al*., 2001).

2.16 Use of *Opuntia elatior* in the treatment of anemia:

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 SP *et al*., 2014).

2.17 Neuroprotective property of *Opuntia elatior*:

The flavonoids like quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether were isolated from ethyl acetate fractions of the fruits and stems of *O. ficus-indica* were evaluated for their protective effects against oxidative neuronal injuries induced in primary cultured rat cortical cells and their antioxidant activities were studied using LPO, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and xanthine oxidase (XO) bioassays. Quercetin was found to inhibit H2O2- or xanthine (X)/xanthine oxidase (XO)-induced oxidative neuronal cell injury, with an estimated IC50 of 4–5 µg/ml and no more protection at concentrations of 30 µg/ml and above, while (+)-dihydro quercetin concentration-dependently inhibited oxidative neuronal injuries, but it was less potent than quercetin. On the other hand, quercetin 3-methyl ether potently and dramatically inhibited H2O2- and X/XO-induced neuronal injuries, with IC50 values of 0.6 and 0.7 µg/ml, respectively. In addition, quercetin and quercetin 3-methyl ether were shown to inhibit XO activity in vitro, with IC50 valuesof10.67and42.01µg/ml, respectively , and quercetin3-methyl ether appears to be the most potent neuroprotectant of the three flavonoids isolated from this plant (Cho J *et al*., 2003).

The methanol extract of *O. ficus-indica* (MEOF) was examined for its neuroprotective action against neuronal injury induced by N-methyl-d-aspartate (NMDA), kainate (KA), and oxygen–glucose deprivation (OGD) in cultured mouse cortical cells and was also evaluated for its protective effect in the hippocampal CA1 region against neuronal damage evoked by global ischemia in gerbils. Treatment of neuronal cultures with MEOF (30, 300, and 1000 µg/ ml) inhibited the NMDA (25 µM), KA (30 µM), and OGD (50 minutes) induced neurotoxicity dose-

dependently. The butanol fraction of *O. ficus-indica* (300µg/ml)significantly reduced NMDA(20µM)induced delayed neurotoxicity by 27%. Gerbils were treated with MEOF every 24 hours for 3 days (0.1, 1.0, and 4.0 g/kg, p.o.) or for 4 weeks (0.1 and 1.0 g/kg, p.o.), and ischemic injury was induced after the last dose. Neuronal cell damage in the hippocampal CA1 region was evaluated quantitatively on the fifth day after the ischemic injury. When gerbils were given doses of 4.0 g/kg (3 days) and 1.0 g/kg (4 weeks), the neuronal damage in the hippocampal region was reduced by 32 and 36%, respectively. These results suggested that the preventive administration of *O. ficus-indica* extracts may be helpful in alleviating the excitotoxic neuronal damage induced by global ischemia (Kima JH *et al*., 2006).

2.18 Chemopreventive effect of cactus *Opuntia ficus-indica* on oxidative stress and genotoxicity of aflatoxin B1:

Aflatoxin B1 (AFB1) is potent hepatotoxic and hepatocarcinogenic agent. In aflatoxicosis, oxidative stress is a common mechanism contributing to initiation and progression of hepatic damage. The aim of this work was to evaluate the hepatoprotective effect of cactus cladode extract (CCE) on aflatoxin B1-induced liver damage in mice by measuring malondialdehyde (MDA) level, the protein carbonyls generation and the heat shock proteins Hsp 70 and Hsp 27 expressions in liver. Protective effect against AFB1-induced genotoxicity also looked for as determined by chromosome aberrations test, SOS Chromotest and DNA fragmentation assay.

Results clearly showed that AFB1 induced significant alterations in oxidative stress markers. In addition, it has a genotoxic potential and it increased the expression of pro apoptotic proteins p53 and bax and decreased the expression of bcl2.

The treatment of CCE before or after treatment with AFB1, showed

i. a total reduction of AFB1 induced oxidative damage markers,
ii. an anti-genotoxic effect resulting in an efficient prevention of chromosomal aberrations and DNA fragmentation compared to the group treated with AFB1 alone
iii. restriction of the effect of AFB1 by differential modulation of the expression of p53 which decreased as well as its associated genes such as bax and bcl2.

CCE have a hepatoprotective effect against aflatoxicosis in mice, probably acting by promoting the antioxidant defence systems (Brahmi D *et al*., 2011).

2.19 Anticancer properties of extracts from *Opuntia humifusa* against human cervical carcinoma cells:

Total polyphenol and ascorbic acid levels in the fruit of *Opuntia humifusa* are higher than those in other parts of the plant. We further hypothesized that antioxidants in *O. humifusa* might affect the growth or survival of cancer cells. Hexane extracts of seeds and ethyl acetate extracts of fruits and stems significantly suppressed the proliferation of HeLa cervical carcinoma cells, but did not affect the proliferation of normal human BJ fibroblasts. Additionally, the extracts of *O. humifusa* induced G1 phase arrest in HeLa cells. The *O. humifusa* extracts reduced the levels of G1 phase-associated cyclin D1, cyclin-dependent kinase 4 (Cdk4), and phosphorylated retinoblastoma proteins. Moreover, p21(WAF1/Cip1) and p53 expression significantly increased after treatment. Effects of ethyl acetate extracts of *O. humifusa* fruit (OHF) become examined on HeLa cells xenograft tumor growth. OHF treatment significantly reduced tumor volume and this decrease was correlated with decreased Cdk4 and cyclin D1 expression. Furthermore, flavonoids, trans Taxifolin, and dihydrokaempferol, were isolated from OHF. Thus, this extract may be a promising candidate for treating human cervical carcinoma (Hahm SW *et al*., 2015).

2.20 Evaluation of antileukemic activity of ripe fruit of *Opuntia elatior*:

Natural food colors such as betanin can inhibit the cell proliferation of a variety of human tumor cells. *Opuntia elatior* Mill. fruit was selected for its *in vitro* antileukemic activity. 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. Its cytotoxic evaluation showed a potent action against to K-562 (Human chronic myelocytic leukemia) cell line. It was found that hydro alcoholic extract of the fruits of *Opuntia elatior* Mill. have persuasive antioxidant activity and promising antileukemic activity (Itankar P *et al*., 2012).

2.21 Toxicology of *Opuntia elatior*:

2.21.1 Acute Toxicity of *Opuntia ficus-indica* and *Pistacia Lentiscus* Seed Oils in Mice:

Opuntia ficus indica and *Pistacia lentiscus L.* seeds are used in traditional medicine. The objective of this study was to investigate the toxicity of the fixed oil of *Opuntia ficus indica* and *Pistacia lentiscus L.* seeds in mice through determination of LD_{50} values, and also the physicochemical characteristics of the fixed oil of these oils. The acute toxicity of their fixed oil were also investigated in mice using the method of Kabba and Berhens. The fixed oil of *Pistacia lentiscus* and *Opuntia ficus indica* seeds were extracted and analyzed for its chemical and physical properties such as acid value, free fatty acid percentage (% FFA), iodine index, and saponification value as well as refractive index and density. LD_{50} values obtained by single doses, orally and intraperitoneally administered in mice, were respectively 43 ± 0,8 ;[40.7– 45.4] ml/kg body wt. p.o. and 2.72 ± 0,1 ;[2.52–2.92] ml/kg body wt. i.p. for *Opuntia ficus indica* ; and 37 ± 1 ;[34.4 − 39.8] ml/kg body wt. p.o. and 2.52 ± 0,2 ;[2.22 − 2.81] ml/kg body wt. i.p. for *Pistacia lentiscus* respectively. The yields of seed oil were respectively calculated as 20.25% and 10.41%. The acid and free fatty acid values indicated that the oil has a low acidity. All doses, orally and intraperitoneally administered, caused immediate agitation and behavioral perturbations with temporary writhing, followed by a quiet attitude period and sedation. Generally, diarrhea was observed and the animals died 12 hours after the administration of fixed oil. Autopsied dead animals showed congested lungs and hearts stopped in diastole. The surviving animals quickly recovered their normal activity and growth, after a period ranging from 4 to 8 days, depending on the dose and mode of administration.

The current study has shown a low toxicity of *Opuntia ficus indica and Pistacia lentiscus* fixed oil. The high values of oral and intraperitoneal lethal doses of both *O. f. indica* fixed oil (LD_{50} value = 43 ml/kg body wt., p.o. ; LD_{50} value = 2.72 ml/kg body wt., i.p.); and *P. lentiscus* fixed oil (LD_{50} value = 37 ml/kg body wt., p.o. ; LD_{50} value = 2.52 ml/kg body wt., i.p.) show their low acute toxicity (Boukeloua A *et al*., 2012).

2.21.2 Hypotensive activity, toxicology and histopathology of opuntioside-I and methanolic extract of *Opuntia dillenii*:

Methanolic extract of *Opuntia dillenii* cladodes and its pure compound alpha-pyrone glycoside, opuntioside-I showed potent hypotensive activity in normotensive rats. Both the extract and opuntioside-I showed comparable effect of 44-54% fall in Mean Arterial Blood Pressure (MABP) at the dose of 10 mg/kg. No mortality was observed in rats even at the doses of 1000 mg/kg/d and 900 mg/kg/d per oral of extract and opuntioside-I respectively. However, histopathology revealed adverse effects of high doses on liver and spleen of the experimental animals (Saleem R *et al*., 2005).

CHAPTER THREE

METHODS AND MATERIALS

3.1 Collection and preparation of plant material:

Plant sample of *Opuntia elatior* was collected from Noakhali in March, 2015. Then proper identification of plant sample was done by an expert taxonomist. The plant was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried plant was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Extraction of the plant material:

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

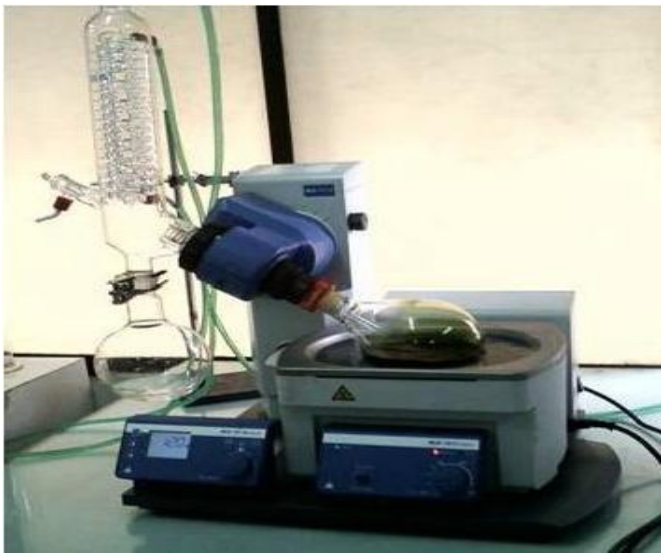


Figure 3.1: Drying of extract using rotary evaporator

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

3.3 Preparation of Mother Solution:

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

3.4 Partition of Mother Solution:

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

3.4.1 Partition with Pet-ether:

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

3.4.2 Partition with Dichloromethane:

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

3.4.3 Partition with Ethyl acetate:

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

3.4.4 Partition with Aqueous Fraction:

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

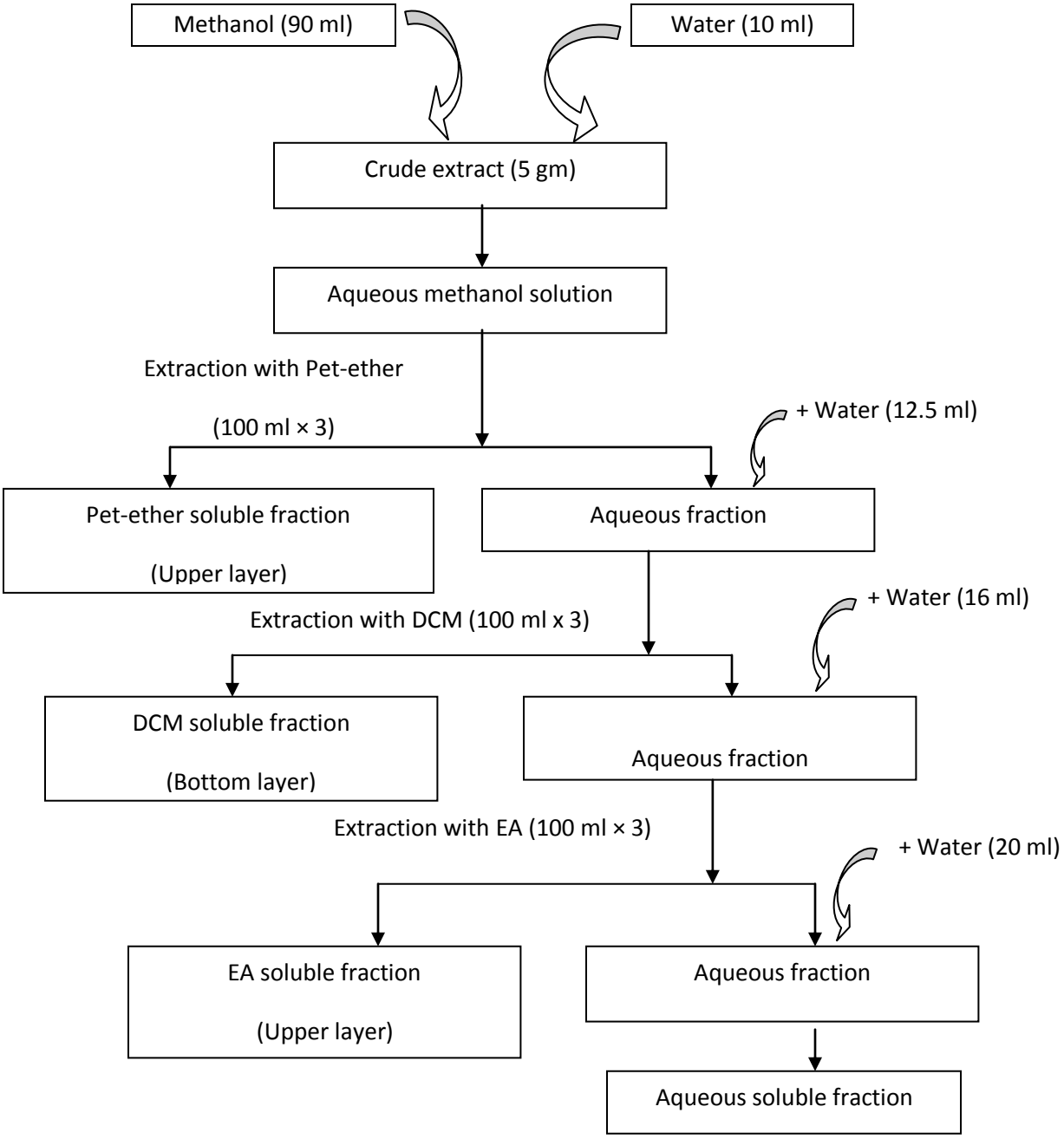


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Opuntia elatior*

3.4.5 Collection of Aqueous Fraction:

After partitioning the mother solution with the four different solvents the aqueous fraction of them were collected and air dried. This aqueous was further investigated for different pharmacological properties such as Antioxidant and Cytotoxic (Beckett AH and Stenlake JB, 1986).

3.5 Brine Shrimp Lethality Bioassay:

3.5.1 Principle:

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (*in-vivo*) lethality, a simple zoological organism, (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested 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 *et al.*, 2013).

3.5.2 Apparatus & Reagents:

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

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

3.5.3 Procedure:

3.5.3.1 Preparation of Sea Water:

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

3.5.3.2 Hatching of Brine Shrimp:

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

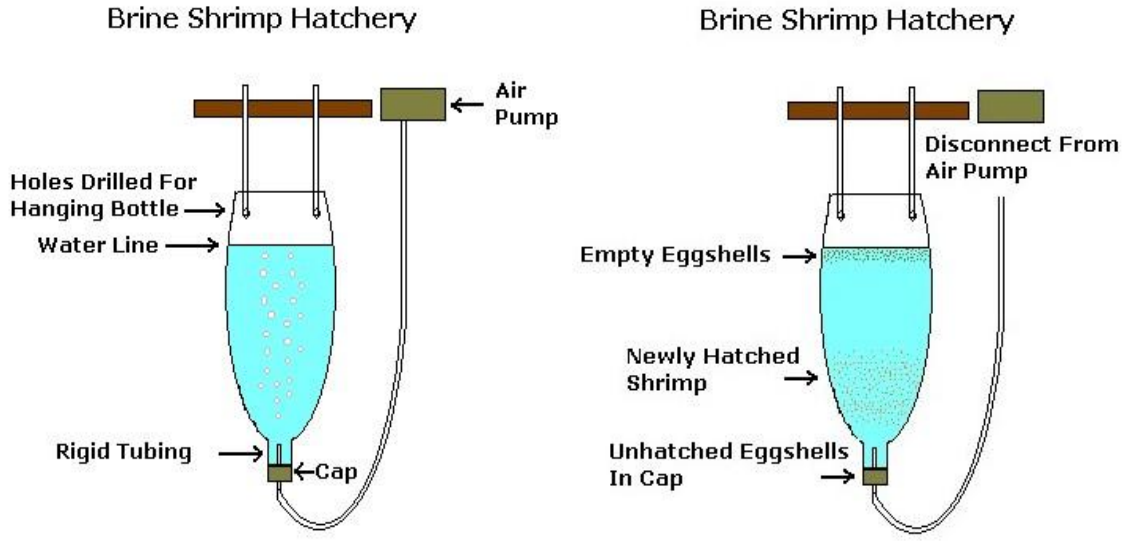


Figure 3.3: Brine shrimp Hatchery

3.5.3.3 Preparation of Test Solutions:

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

3.5.3.4 Preparation of the Test Samples of Experimental Plant:

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

3.5.3.5 Preparation of the Positive Control Group:

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

3.5.3.6 Preparation of the Negative Control Group:

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

3.5.3.7 Counting of Nauplii:

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

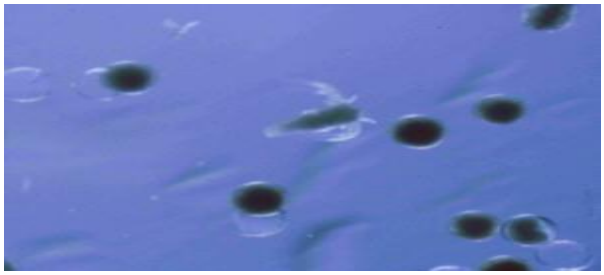


Figure 3.4: Counting of nauplii

3.6 Antioxidant Activity:

3.6.1 Total Phenolic Content:

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

3.6.1.1 Principle:

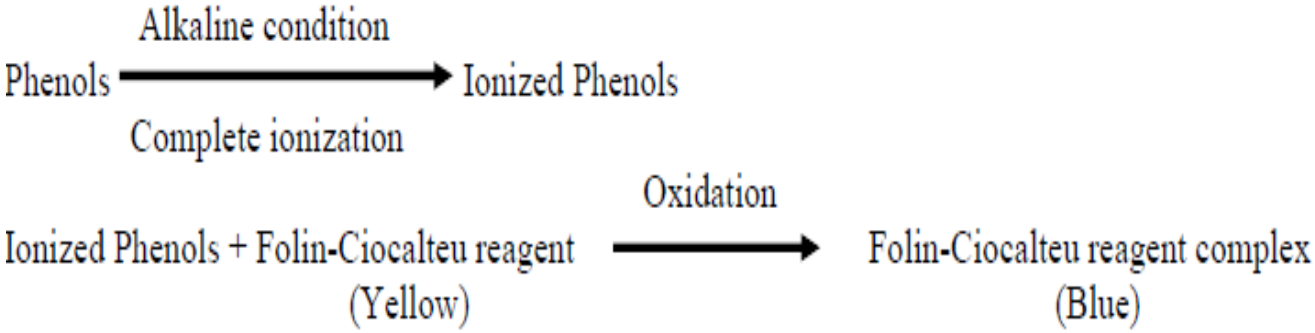
The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. In the alkaline condition phenols ionize completely.

Table 3.2: Composition of 100 mg Folin-Ciocalteu Reagent

i.	Water	57.5 ml
ii.	Lithium Sulfate	15.0 mg
iii.	Sodium Tungstate Dihydrate	10.0 mg
iv.	Hydrochloric Acid (25%)	10.0 mg
v.	Phosphoric Acid 85% solution in water	5.0 mg
vi.	Molybdic Acid Sodium Dihydrate	2.5 mg

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain 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).



3.6.1.2 Apparatus & Reagents:

Table 3.3: Apparatus and reagents used for total phenolic content

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

3.6.1.3 Procedure:

Standard curve preparation: Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120 µg/ml to 80 µg/ml. 5 ml of FCR (diluted 10 times with water) and 4 ml of Na_2CO_3 (7.5% w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

Sample preparation: 2 mg of the *Opuntia elatior* aqueous fraction was taken and dissolved in 1 ml methanol to get a sample concentration of 2 mg/ml.

Determination of total phenol content:

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

3.6.2 Total Flavonoid Content:

3.6.2.1 Principle:

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

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

3.6.2.2 Apparatus & Reagents:

Table 3.4: Apparatus and reagents used for total flavonoid content

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

3.6.2.3 Procedure:

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

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

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

Preparation of Standard Solution: The stock solution was prepared by taking 0.025 gm of ascorbic acid and dissolved into 5 ml of ethanol. Concentration of this solution was 5000 µg/ml of ascorbic acid. The experimental concentrations were prepared from this stock solution.

Table 3.5: Preparation of standard solution

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

Preparation of Extract Solution: 5 ml of each plant extracts were taken and dissolved into 5 ml of methanol. The concentration of the solution was 1 mg/ml of plant extracts. Then the following steps were carried out. 1.5 ml extract was taken in a test tube and then 6 ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for 6 minutes. 4% NaOH and 0.6 ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5 ml methanol was taken and same procedure was repeated.

Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank.

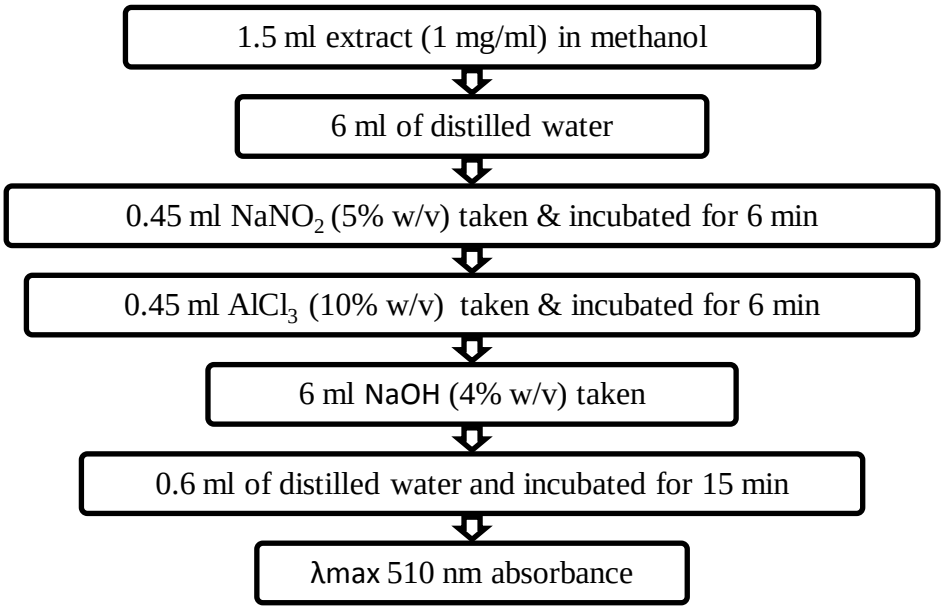


Figure 3.5: Schematic diagram of preparation of extract solution

Preparation of blank solution:

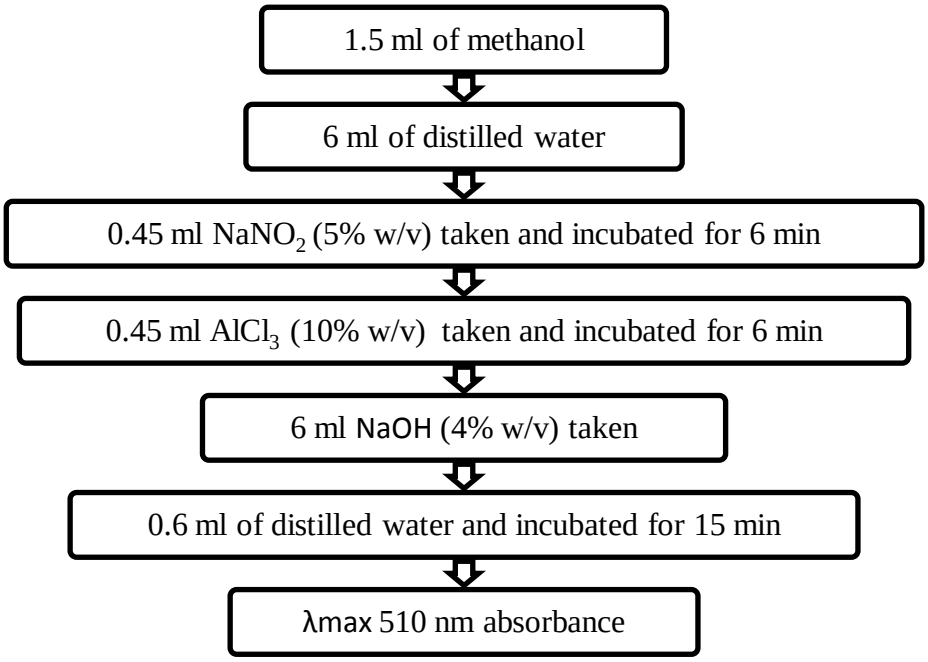


Figure 3.6: Schematic diagram of preparation of blank solution (Zhishen J *et al*., 1999).

3.6.3 Reducing power assay:

3.6.3.1 Principle:

The reducing power of petroleum ether extract of *Opuntia elatior* was 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.

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

3.6.3.2 Apparatus and reagents:

Table 3.6: Apparatus and reagents used for Reducing power assay

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

3.6.3.3 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:

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

Trichloro acetic acid (10%) preparation:

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

Ferric chloride (0.1%) preparation:

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

Standard solution preparation:

The stock solution was prepared by taking 0.025 gm of ascorbic acid and dissolved into 5 ml of methanol. The concentration of this solution was 5000 µg/ml of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.7: Different concentrations of ascorbic acid solution preparation

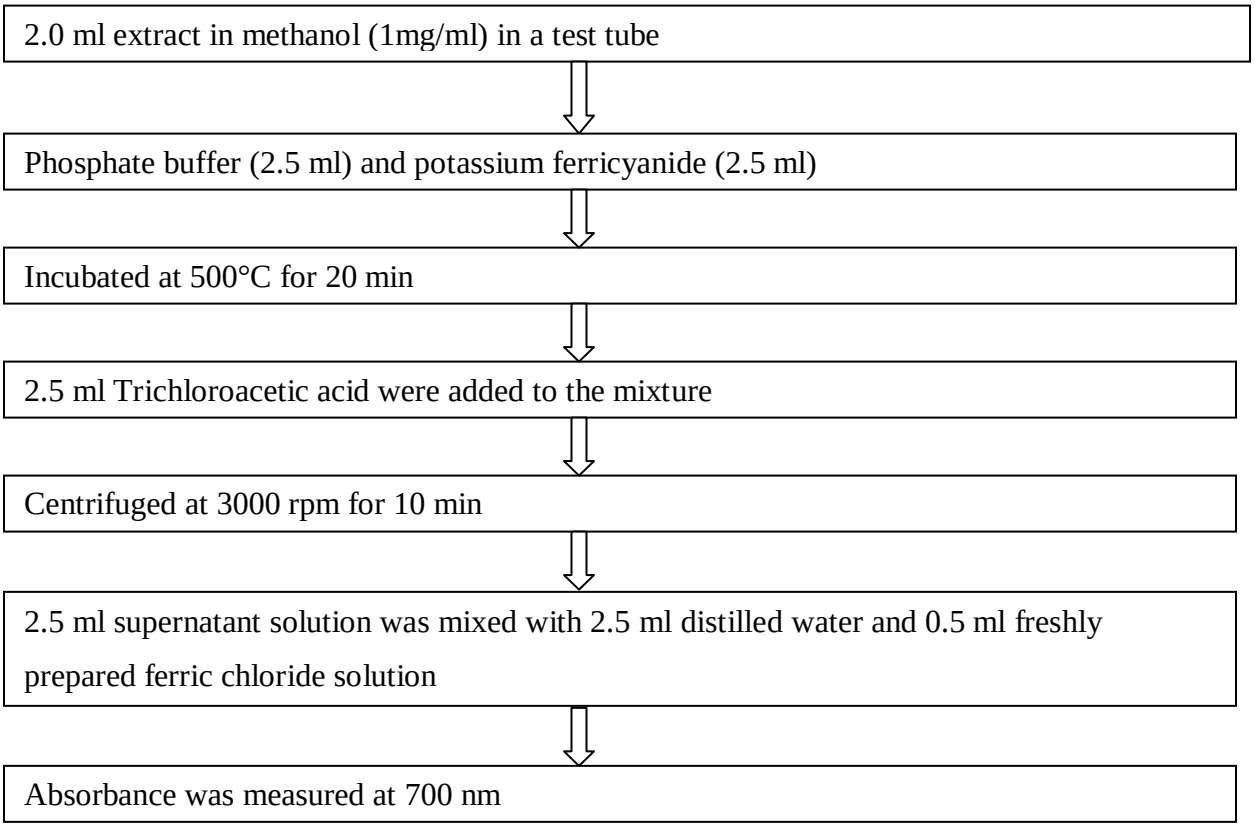
Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

Extract solution preparation:

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

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.



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

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 (Oyaizu M, 1986).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Result of Brine Shrimp Lethality Bio-Assay:

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

The percentage mortality at each concentration was determined using the following formula:

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

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

4.1.1 Preparation of Curve for Standard:

Here, Tamoxifen was used as reference standard.

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

Test tube number	**Concentration (µg /ml)**	**Log C**	**Number of alive nauplii**	**Number of dead nauplii**	**% Mortality**	**LC_{50} (µg /ml)**
1	400	2.602	0	10	100	25.00
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	

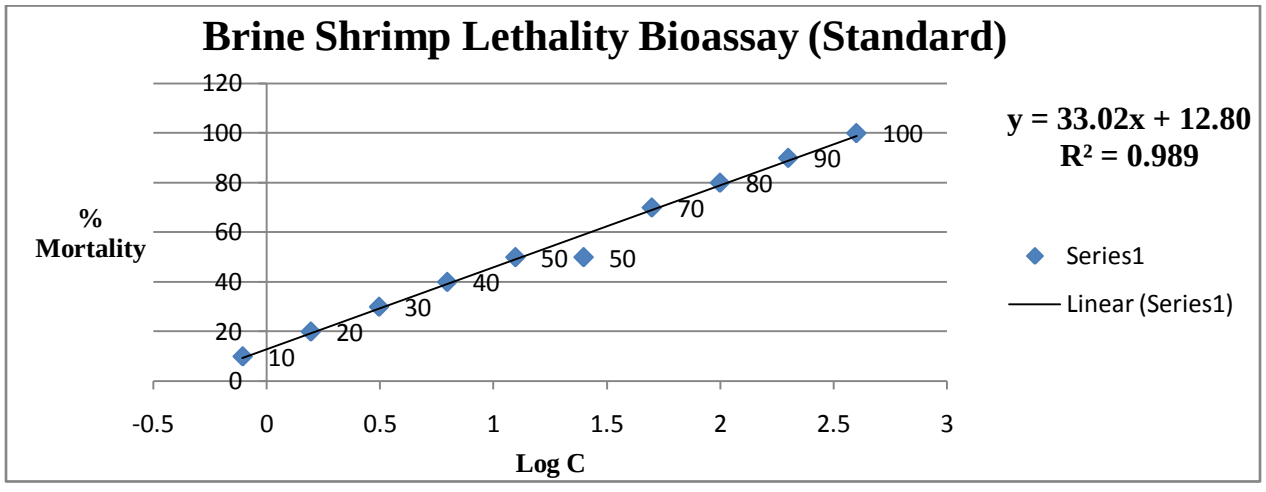


Figure 4.1: % Mortality and Predicted Regression Line of Tamoxifen (standard)

4.1.2 Preparation of Aqueous Fraction Curve of *Opuntia elatior*:

Table 4.2: Results of the bioassay in aqueous fraction of *Opuntia elatior*

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	2	8	80	12.5
2	200	2.301	3	7	70	
3	100	2.000	3	7	70	
4	50	1.699	4	6	60	
5	25	1.398	4	6	60	
6	12.5	1.097	5	5	50	
7	6.25	0.796	5	5	50	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	0.78125	-0.107	8	2	20	

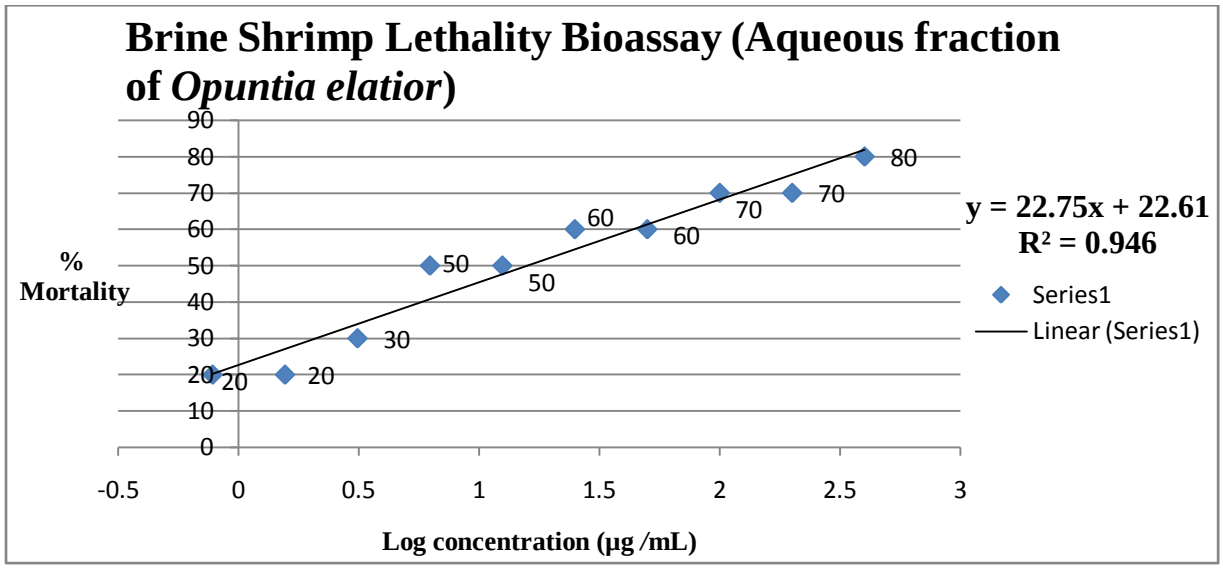


Figure 4.2: % Mortality and Predicted Regression Line in aqueous fraction of *Opuntia elatior*

4.1.3 Discussion:

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional to the concentration. 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 4.1 and Table 4.2.

Table 4.3: Cytotoxic activity of Tamoxifen and aqueous fraction of *Opuntia elatior*

Sample	**Linear regression equation**	**R^2 value**	**LC_{50} (µg/ml)**
Standard (Tamoxifen)	y = 33.02 x + 12.80	0.989	25.00
Aqueous fraction	y = 22.75 x + 22.61	0.946	12.50

In this investigation, standard and aqueous fraction exhibited cytotoxic activities with the LC_{50} values 25 µg/ml and 12.50 µg/ml respectively as shown in Table 4.3. LC_{50} value of *Opuntia elatior* in aqueous fraction showed more activity of it than Tamoxifen. Further investigation is needed to confirm the activity.

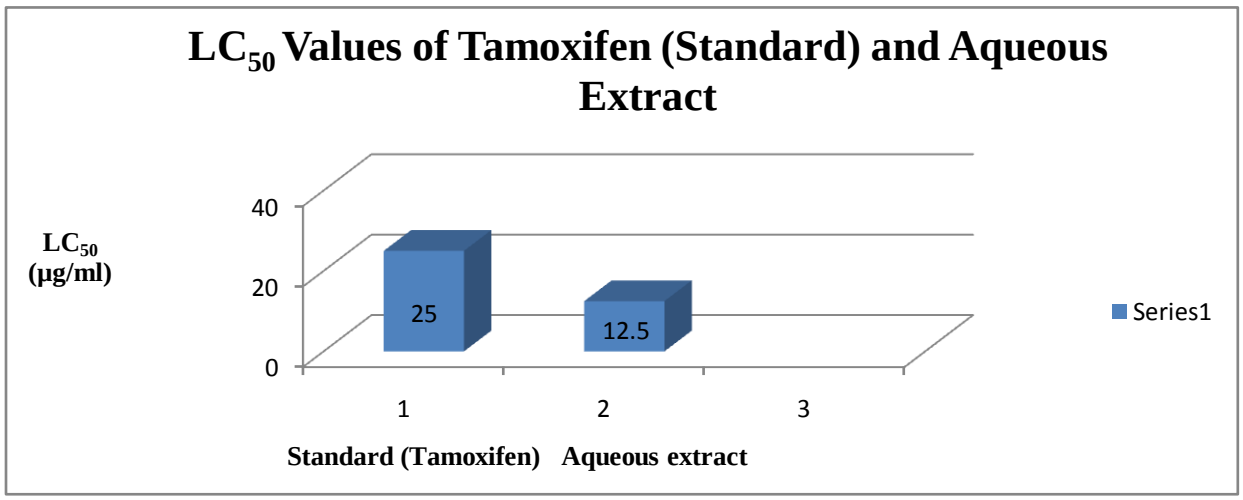


Figure 4.3: Comparison between LC_{50} values of standard and extract

4.2 Result of Antioxidant Tests:

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

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

4.2.1 Result of Total Phenolic Content:

The aqueous extract of *Opuntia elatior* and the aqueous fractions of the methanol extract of *Opuntia elatior* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard (Singleton *et al.*, 1999).

4.2.1.1 Preparation of Standard Curve:

Table 4.4: Total Phenolic content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 765 nm)**	**Regression line**	**R^2 value**
80	2.406	y = 0.019 x + 0.824	0.937
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 4.4. This linear curve was considered as a standard curve.

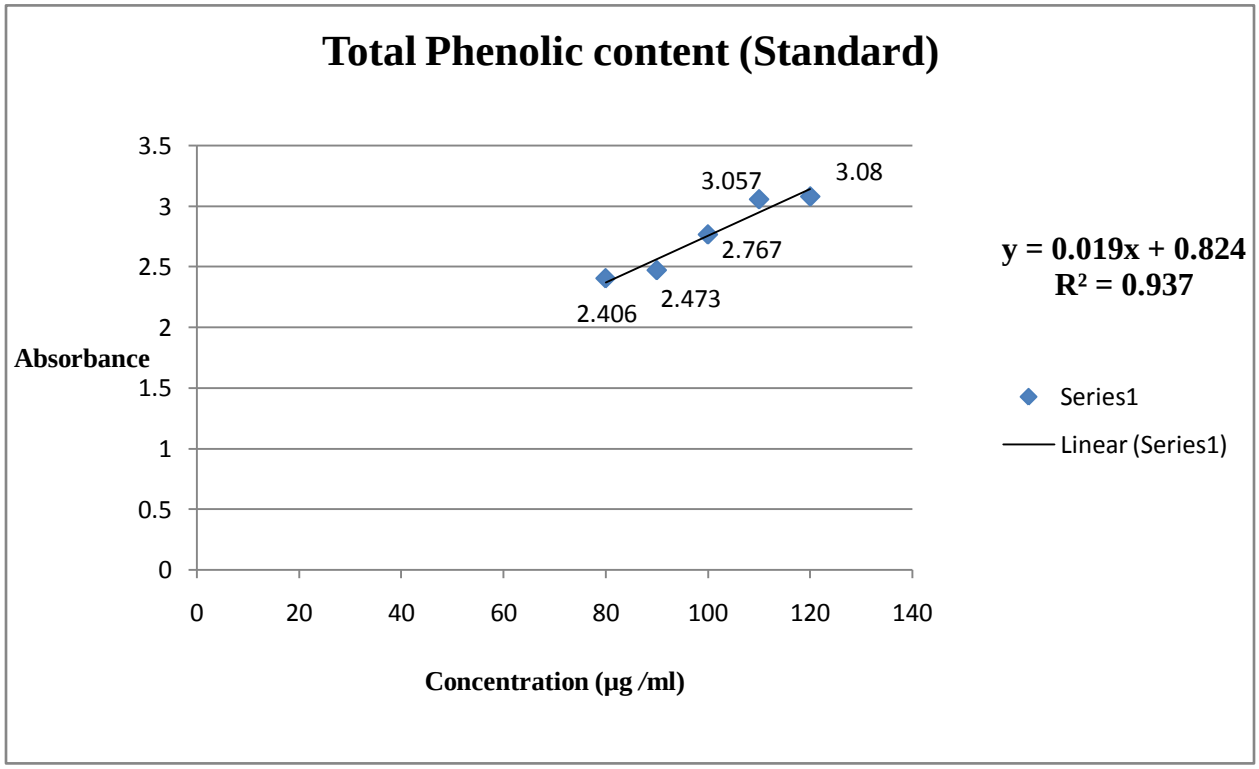


Figure 4.4: Graphical representation of Phenolic content of ascorbic acid

4.2.1.2 Total Phenolic content present in aqueous extract of *Opuntia elatior*:

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

Table 4.5: Total Phenolic content in aqueous fraction of *Opuntia elatior*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 765 nm)**	**Total Phenolic (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Opuntia elatior*	2	1.292	12.315

4.2.1.3 Discussion:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the aqueous fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 12.315 mg of AAE/gm of dried extract of phenol content was found in the aqueous fraction of *Opuntia elatior*.

4.2.2 Result of Total Flavonoid Content:

The aqueous fractions of *Opuntia elatior* were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard (Chang C *et al.*, 2002).

4.2.2.1 Preparation of Standard Curve:

Table 4.6: Total Flavonoid content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 510 nm)**	**Regression line**	**R^2 value**
50	0.05	y = 0.001x-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 4.5. This linear curve was considered as a standard curve.

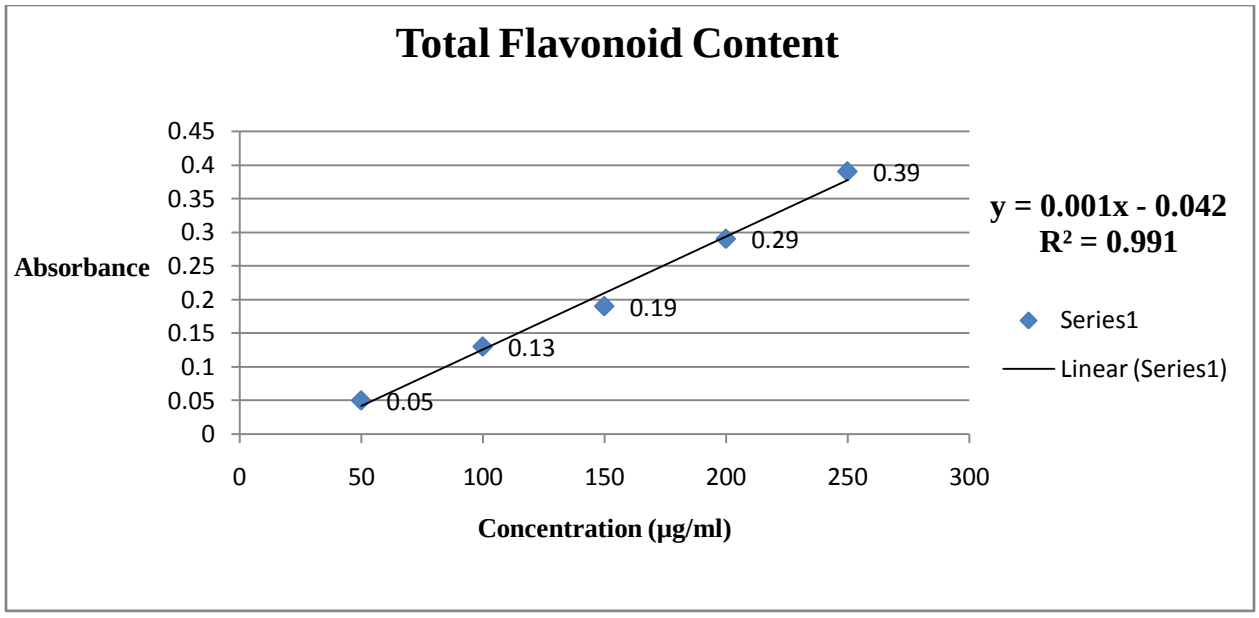


Figure 4.5: Graphical representation of Flavonoid content of ascorbic acid

4.2.2.2 Total Flavonoid Content Present in aqueous fraction of *Opuntia elatior*:

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

Table 4.7: Total Flavonoid content in aqueous fraction of *Opuntia elatior*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 510 nm)**	**Total Flavonoid (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Opuntia elatior*	1	0.014	56

4.2.2.3 Discussion:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in flavonoid content. Absorbance of the aqueous fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 56 mg of AAE/gm of dried extract of flavonoid content was found in the aqueous fraction of *Opuntia elatior*.

4.2.3 Result of Total Reducing Power Assay:

The aqueous extract of *Opuntia elatior* and the aqueous fractions of the methanol extract of *Opuntia elatior* were subjected to determine total reducing power. Ascorbic acid was used as reference standard (Oyaizu M, 1986).

4.2.3.1 Preparation of Standard Curve:

Table 4.8: 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	R^2 = 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 4.6. This linear curve was considered as a standard curve.

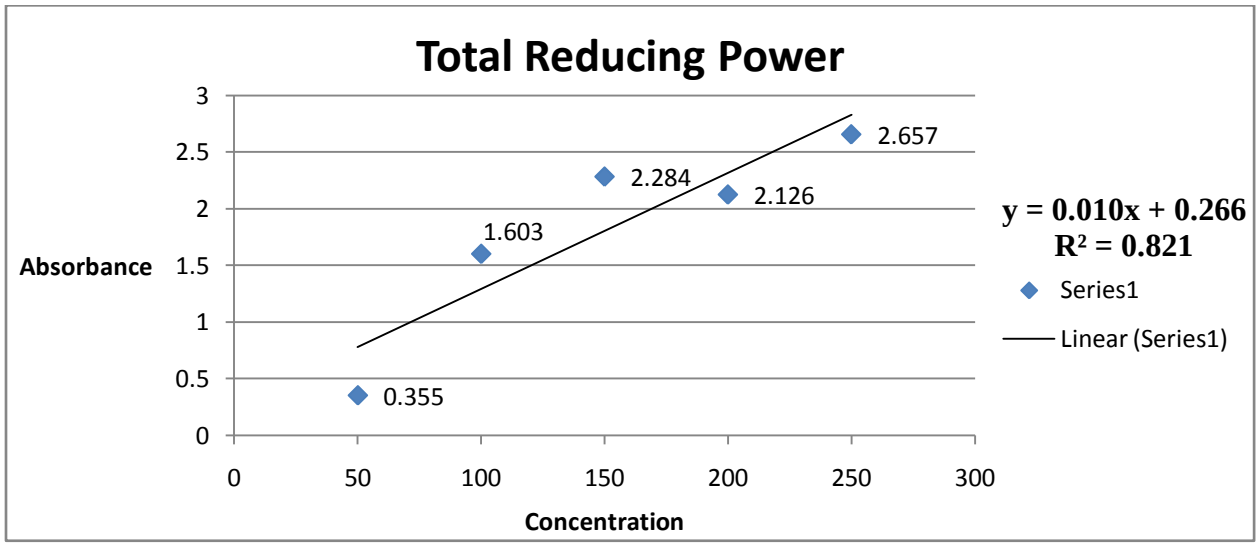


Figure 4.6: Graphical representation of Reducing power of ascorbic acid

4.2.3.2 Total Reducing Power Assay in aqueous extract of *Opuntia elatior*:

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 extract is calculated and given in the table below.

Table 4.9: Total reducing power in aqueous fraction of *Opuntia elatior*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 700 nm)**	**Total reducing power (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Opuntia elatior*	1	0.571	30.5

4.2.3.3 Discussion:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in reducing power content. Absorbance of the aqueous fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 30.5 mg of AAE/gm of dried extract of reducing power content was found in the aqueous fraction of *Opuntia elatior*.

CHAPTER FIVE

CONCLUSION

Conclusion:

As the literature review suggests, the presence of several phytochemical compounds in aqueous fraction of *Opiuntia elatior*, makes the plant pharmacologically active.

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

Antioxidant property in aqueous extract of *Opuntia elatior* was determined by Phenolic content assay, Flavonoid content assay and Reducing power assay. Phenolic content was 12.315 mg/gm, Flavonoid content was 56 mg/gm and Reducing power was 30.5 mg/gm in aqueous extract of *Opuntia elatior.* So aqueous extract of *Opuntia elatior* have poor antioxidant property. Mixture of compounds can lower antioxidant property in aqueous fraction of *Opuntia elatior,* if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of aqueous fraction of *Opuntia elatior.*

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

CHAPTER SIX

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