Cytotoxic and antioxidant evaluation of methanolic extract of *Aglaonema hookerianum*

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY

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Declaration By The Research Candidate

I, Samin Yasar Arnob, hereby declare that the dissertation entitled "**Cytotoxic and antioxidant evaluation of methanolic extract of *Aglaonema hookerianum***" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me, under the supervision and guidance of Nazia Hoque, Senior Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Endorsement By The Chairperson

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Dedication

This Research Paper is dedicated to

My beloved parents and

My beloved teachers

Abstract

Aglaonema hookerianum is an herb found in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. The species is traditionally used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus and in stomachache, conjunctivitis and constipation by various communities of the region. The objective of the study was to evaluate the methanolic extract of *Aglaonema hookerianum* of whole plant for antioxidant activity and cytotoxic activity. The antioxidant activity of crude methanolic extract of *Aglaonema hookerianum* was evaluated by reducing power assay method. The color change was determined with the UV spectrophotometer. Values of Concentration against Absorbance was plotted to prepare a curve for the extract and standard ascorbic acid. The results indicate that as the concentration increases, absorbance increases proportionately. When the results are compared with the standard, the increase in reducing power is slightly less than the standard. This suggests the presence of bioactive compounds having antioxidant activity but is not potent at the required concentration. The crude methanolic extract of *Aglaonema hookerianum* was also screened for cytotoxic activity using brine shrimp lethality bioassay. The nauplii were treated with different concentration of the extract and the standard Tamoxifen. From the curve of % Mortality against Log of Concentration, LC_{50} value was determined. The LC_{50} value of Tamoxifen and the extract was 13.38 μg/ml and 44.8 μg/ml respectively which indicates the extract is less potent than the standard but possesses significant cytotoxic activity as substances less than 1000 μg/ml is presumed to possess cytotoxic activity.

Keyword: *Aglaonema hookerianum*, Cytotoxic assay, Antioxidant assay, Brine shrimp lethality bio-assay,

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Chapter One

INTRODUCTION

1.1 Introduction

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new modus vivendi of the industrialized western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value (Rates, 2001).

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank et al., 1982; Vulto and Smet, 1988; Mentz and Schenkel, 1989). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless (Rates, 2001).

Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, civilization turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported.

Ethnobotany, the scientific study of the relationships that exist between humans and plants, is a recognized way to discover new effective medicines for future and further use. In ancient Greece, plants were classified and descriptions of them were given by scholars. It aids in the identification process. Researchers identified in 2001, 122 compounds that were isolated and identified from "ethno medical" plant sources, are used in modern medicine. The current use of the active elements of the plants is 80% similar to those of ethno medical use (Fabricant and Farnsworth, 2001).

1.1.1 Medicinal Plants as Drugs

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal and human body are generally designated as medicinal plants (Ghani, 1998).

Or, according to the World Health Organization (WHO),

"A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis" (Sofowara, 1982).

The history of the use of medicinal plants for alleviating diseases had its origin in the activities of the most primitive man of the remote past (Ghani, 1998). Our ancestors were forced to use any natural substances that they could find to ease their sufferings caused by acute and chronic illness, physical discomforts, wounds and injuries and even terminal illness. Since that ancient time, plants with therapeutic properties have occupied an important place in the disease treatment practices (Khan et al., 2005).

Concerning drugs of plant origin, it is important to bear in mind certain conceptual distinctions. Plants can be used as therapeutic resources in several ways. They can be used as herbal teas or other home-made remedies, when they are considered as medicinal plants. They can be used as crude extracts or "standard enriched fractions" in pharmaceutical preparations, such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phytopharmaceutical preparations or herbal medicines.

Finally, plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest, which can themselves be active and used directly as a drug, examples being quinine, digoxin and ergotamine, or they can be used as precursors (e.g. diosgenin) in semi-synthetic processes or as models for total synthesis, with well-defined pharmacological activity or structure–activity relationship studies determining a prototype drug (e.g. morphine) (Rates, 2001).

1.1.2 Medicinal plants from Ancient Times

Archaeological evidence indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years, to the Sumerians, who created lists of plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs. The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Seeds likely used for herbalism have been found in the archaeological sites of Bronze Age China dating from the Shang Dynasty. Over a hundred of the 224 drugs mentioned in the Huangdi Neijing, an early Chinese medical text, are herbs. Herbs were also common in the medicine of ancient India, where the principal treatment for diseases was diet. *De Materia Medica* by Pedanius Dioscorides, a Roman physician, is a particularly important example of such writings. The documentation of herbs and their uses was a central part of both Western and Eastern medical scholarship through to the 1600s, and these works played an important role in the development of the science of botany (Nunn, 2002; Robson et. al., 2009, Hong, 2004; Ackerknecht, 1982).

Human beings have used plants for the treatment of diverse ailments for thousands of years. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements, since they

cannot afford the products of Western pharmaceutical industries, together with their side effects and lack of healthcare facilities. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Ernst, 2007).

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Ernst, 2007). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Fabricant and Farnsworth, 2001).

1.1.3 Traditional medicine

Traditional medicines have existed in Bangladesh as an important basis of health care since olden times. Because of their potentialities and close association with the culture and tradition of the people, traditional systems of medicine have assumed a unique position in the health care of the people living in even the remotest areas of the country. Although the use of traditional medicine is so deeply rooted in the cultural heritage of

Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts (Samy, Pushparaj and Gopalakrishankone, 2008). Among the largest ethnic group, the bangles on the main land, there are two distinct forms of Traditional medicine practice (Ghani, 1998):

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

- *Folk medicine***,** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting , bonesetting, hot and cold baths, therapeutic fasting and cauterization.
- *Religious medicine,* which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc. and
- *Spiritual medicine***,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

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

- *Unani-Tibb or Graeco-Arab system***,** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and
- *Ayurvedic system***,** which is the old Indian system, based on the *Vedas* the oldest scriptures of the Hindu saints of the Aryan age.

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion

of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them (Ghani, 1998).

As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field (Samy, Pushparaj and Gopalakrishankone, 2008). Some medicinal uses of common plants in Bangladesh are reported in following table.

Table 1: Name and medicinal uses of some common plants in Bangladesh (Samy, Pushparaj and Gopalakrishankone, 2008)

Common name	Botanical name	Parts Used	Uses
Pudina	*Menthe arvensis*	Whole plant	Indigestion, stomach disorder, stimulant.
Kalmegh/ Bhui neem	*Andrographis paniculata*	Whole plant	Fever, Weakness, Release of gas.
Kalmishak	*Smilax zeylanica*	Roots, steams	blood dysentery, rheumatisms, abscess
Dhutara	*Datura metel*	Roots, leaves, seeds	Anesthesia, pain, asthma, epilepsy, rheumatic fever, hypertension.
Tulsi	*Ocimum sanclum*	Leaves, flower, seeds	Cough, Cold, Bronchitis, Expectorant.
Henna/Mehdi	*Lawsennia iermis*	Leaves, flower	Burning, Steam, Anti-inflammatory
Gritkumari	*Aloe verra*	Leaves	Laxative, Wound healing, Skin burns & care, Ulcer.
Anantamul/sariva	*Hemidesmus indicus*	Root, leaves	Appetizer, Carminative, Aphrodisiac, Astringent
Sharisa	*Brassica napus*	Leaves, seeds.	Fever, common cold, stomachache, itching, headache.
Vringraj	*Eclipta alba*	Whole plant	Anti-inflammatory, Digestive, Hair tonic
Neem	*Azardirchata indica*	Leaves	Sedative, Analgesic, Epilepsy,Hypertensive

1.1.4 Significances of Medicinal Plants to Mankind

Even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong Shu, 1998). The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann, 1991). In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Williamson et al., 1996).

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

• Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

• Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

• Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.

1.1.5 Advantages of Drug Discovery from Natural Resources

Usage of botanical sources as starting point in the drug development program is associated with few specific advantages:

- Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active com-pounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. At certain time point afterward, one may attempt upon synthesis of active molecule and reduce pressure on the resource. Drug development from *Rauwolfia serpentina*, *Digitalis purpurea*, etc. in the past fall under this category of approach.

- Sometimes, such approaches lead to development of novel molecules derived from the source due to inherent limitations of the original molecule. For instance, podophyllin derived from *Podophyllum hexandrum* was faced with dose-limiting toxicities. Such limitations could be overcome to a great extent by semi-synthesis of etoposide, which continues to be used in cancer therapy today. Similar was the case with camptothecin (originally isolated from *Camptotheca sp*. and subsequently from *Mappia sp*.), which led to development of novel anticancer molecules like topotecan and irinotecan.

- Natural resources as starting point has a bilateral promise of delivering the original isolate as a candidate or a semi-synthetic molecule development to overcome any inherent limitations of original molecule.

1.1.6 Value of Medicinal plants

Plants are valuable for modern medicine in four basic ways:

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

2. They serve as raw materials base for elaboration of more complex semi synthetic chemical compounds.

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

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

1.1.7 Global scenario of Medicinal plants

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal medicine is a common element in Ayurvedic, homeopathic, naturopathic, traditional, oriental, Native American & Indian medicine. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. The present global herbal market is worth about US$ 62 billion per annum. The annual growth of herbal market is about 15 percent and the global herbal market by 2050 is expected to be about US$ 5 trillion (Payyappallimana, 2009).

Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs (Calixto, 2000) have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide (Rates, 2001).

1.1.8 Medicinal plants in Bangladesh

In an estimate, the international market of medicinal plants related to trade stood at 60 billion US Dollar per year. The demand for medicinal plants based raw materials are growing at an approximate rate of 10-15% per year internationally. Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jumping in volume of plants materials trade within and across the country. Bangladesh there is no systematic cultivation process or conservation strategies about medicinal plants. The local people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation. This knowledge is now under threat to extinction. This is a very alarming situation with regard to natural growth of medicinal plants in the wilderness in this country. In this scenario, the survey on "Traditional and industrial use and market Scenario of Medicinal plants in Bangladesh." has been conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh. We have found that there is worth of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products. This research aimed at documenting the 'Present Status and Market Scenario of Medicinal Plants' in Bangladesh. Our research finding shows that 84.1% of the respondent use medicinal plants in health care. 18.3% of the villagers use Kabirazi in the disease in medium category.55.0% of our respondent's source of knowledge of using medicinal plant is family where 34.7% gained knowledge from neighbor. Only 14.3% of the respondents are involved with trading of medicinal plant. About 10.4% of the villagers are involved in cultivation, collection or business of medicinal plant. From the survey report it has been found that 46.6% industries are using above 60% of imported medicinal plants as their raw materials and 53.3% of the industries are using below 40%. The study revealed that 86.7% industries are importing Indian raw materials, 53.3% are importing the Pakistani one and very few of them are importing the raw materials from Nepal, Iran and Korea. According to the response of shop owners, the local raw materials of their products are mostly coming from 5 different areas of the country. Among those 90% are coming from Chittagong and again 76.6% from Tangail, 30% from Gazipur and another

30% from Khulna. In this scenario, appropriate steps must therefore be taken immediately in order to save this situation with regard to growth, conservation and supply of medicinal plants in the country. The best possible way of doing this is to bringing this more and more of these plants under planned cultivation. The cultivation of medicinal plants in Bangladesh will lead to the conservation and also protect the biodiversity. Ecological and biotic factors are suitable in Bangladesh for the cultivation of medicinal plants. We have been successful to sensitize the policy makers. In Bangladesh there is no facilities and skilled manpower for the processing of MPs. Our research is now aiming to develop processing unit and to train the garden owner for skilled manpower to value addition of MP, which will create the income generating women in rural areas.

In Bangladesh, about 500 plant species have been identified as medicinal plants because of their therapeutic properties, Approximately 400 herbal factories have been established in this country for producing Ayurvedic and Unani medicines. It has been estimated that Bangladesh has a market of about 100-core taka worth herbal products annually. The total size of the medicinal plant market at wholesale prices was estimated at some US$ 14 million per annum which corresponds to 17000 tons of products .It has been estimated that 12,500 tonnes of dried medicinal plant products are sold in Bangladesh that have a worth of Tk 255 million to rural economy. At the factory level, 5000 tonnes of medicinal plants are imported annually that cost around 480 million taka (Alam et al., 1996). Although modern medicinal science has been developed to a great extent, many rural people of Bangladesh still depend on plant products and herbal remedies for treating their ailments (Bregum, 2004).

1.2 Bioactive Compounds in Medicinal Plants

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (Bernhoft, 2010)-

(a) Primary metabolites such as sugars and fats, which are found in all plants; and

(b) Secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination.

It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development (Bernhoft, 2010).

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs (Bernhoft, 2010):

- Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimeric galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.
- Polyphenols (also known as phenolics) are compounds contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.
- Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important

roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by an herbivore.

- Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. (The name "terpene" is derived from the word "turpentine"). Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

The goals of using plants as sources of therapeutic agents are (Bernhoft, 2010)-

- to isolate bioactive compounds for direct use as drugs, e.g. digoxin, digitoxin, morphine , reserpine, taxol, vinblastine, vincristine etc
- to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics),

taxotere which are based respectively on galegine, Δ9-tetrahydrocannabinol, morphine, taxol.

- to use agents as pharmacologic tools, e.g., lysergic acid diethylamide, mescaline, and
- to use the whole plant or part of it as a herbal remedy, e.g., cranberry, garlic etc.

Table 2: Drugs derived from Natural products with their Therapeutic use (Mishra and Tiwari, 2011)

Trade name	Lead compound	Therapeutic use
Dronabinol (Sativex™)	Dronabinol	Pain
Fumagillin (Flisint™)	Fumagillin	Antiparasitic
Tigecycline (Tygacil™)	Tetracycline	Antibacterial
Zotarolimus (Endeavor™)	Sirolimus	Cardiovascular
Anidulafungin (Eraxis™)	Echinocandin	Anti-fungal
Exenatide (Byetta™)	Exenatide-4	Diabetes
Lisdexamfetamine (Vyvanse™)	Amphetamine	ADHD
Temsirolimus (Torisel™)	Sirolimus	Oncology
Methylnaltrexone (Relistor™)	Naltrexone	Pain
Telavancin (Vibativ™)	Vancomycin	Antibacterial
Romidepsin (Istodax™)	Romidepsin	Oncology
Monobactam aztreonam (Cayston™)	Monobactam aztreonam	Antibacterial

1.3 Approaches for isolation of active compounds from natural origin

1.3.1 Random approach

Two approaches have been followed for screening of the plants selected randomly for the purpose of new drug discovery (Katiyar et al., 2012)-

a) *Screening for selected class of compounds like alkaloids, flavonoids*, etc.: While this route is simple to perform, however, it is flawed in the sense that it provides no idea of the biological efficacy. However, chances of getting novel structures cannot be denied following this approach.

b) *Screening of randomly selected plants for selected bioassays*: Central Drug Research Institute, a premier R and D organization of Council of Scientific and Industrial Research of India, followed this approach about three decades ago. They screened almost 2000 plants for biological efficacy. However, the screening did not yield any new drug. National Cancer Institute (NCI) of National Institute of Health, USA, studied about 35,000 plant species for anticancer activity, spending over two decades from 1960 to 1980. It resulted in proving two success stories, which were those of paclitaxel and camptothecin. This route, therefore, has been applied for both focused screening as well as general screening, showing some success in focused screening. If target-based bioassays are used, e.g. screening against PTP1B, chances of success would probably be more. This approach, however, needs a huge library of extracts, which very few organizations in the world are having.

1.3.2 Ethnopharmacology approach

The approach of ethnopharmacology essentially depends on empirical experiences related to the use of botanical drugs for the discovery of biologically active New Chemical Entities (NCEs). This process involves the observation, description, and experimental investigation of indigenous drugs, and is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines like anthropology, archaeology, history, and linguistics. This approach based on ethnomedicinal usage history has seen some success, e.g. *Andrographis paniculata*was used for dysentery in ethnomedicine and the compounds responsible for the activity were isolated as andrographolide. Morphine from *Papaver somniferum,* Berberine from *Berberis aristata,* and *Picroside* from Picrorrhiza kurroa are some examples of this approach. Some of the plants which are not selected on the basis of ethnomedical use also had some success stories, like L-Dopa from *Mucuna prurita* and paclitaxel from *Taxus brevifolia* (Katiyar et al., 2012).

1.3.3 Traditional system of medicine approach

Countries like India and China have a rich heritage of well-documented traditional system of medicine in vogue. Though these codified systems of medicine use largely botanical sources as medicines, however, these stand apart from ethnomedicine specifically on three accounts (Katiyar et al., 2012):

- The ethnomedicinal practice is based on empirical experiences. On the other hand, these codified systems built up the empirical practices on strong conceptual foundations of human physiology as well as of pharmacology (though the tools of their investigations in those times were far different from the existing ones).
- The pharmaceutical processes have been more advanced as against the use of crudely extracted juices and decoctions in ethnomedicinal practices. Due to this phenomenon, the concept of standardization was known to the system.
- They are well documented and widely institutionalized. On the other hand, the ethnomedicinal practices are localized and may be largely controlled by few families in each of the community.

However, in terms of historicity, ethnomedicinal practices might be older than codified systems of medicine (Katiyar et al., 2012).

Discovery of artemisinin from *Artemesia alba* for malaria, guggulsterones from *Commiphora mukul* (for hyperlipidemia), boswellic acids from *Boswellia serrata* (anti-inflammatory), and bacosides from *Bacopa monnieri* (nootropic and memory enhancement) was based on the leads from these codified systems of medicine prevailing in China and India. However, it can be stated that such approach for selecting candidates in drug discovery programs has not been adopted much so far. Nonetheless, the approach has a distinct promise in terms of hit rates. But the distinct example for this approach has been the discovery of reserpine from *Rauwolfia serpentine*, which was based on the practices of Unani medicine (Katiyar et al., 2012).

1.3.4 Zoo-pharmacognosy approach

Observation of the behavior of the animals with a view to identify the candidate plants for new drug discovery is not a distant phenomenon. Observation of straight tails linked to cattle grazing habits in certain regions of South America led to identification of a plant *Cestrum diurnum* and three other plant members of family Solanaceae, which probably are the only known plant sources of the derivatives of Vitamin D_3. This approach, however, needs close observation and monitoring of the behavior of animals (Katiyar et al., 2012).

1.4 Procedure for Development

Since drug development is an expensive practice, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required. The way of developing drugs from plants involves several stages (Ghani, 1998), which include:

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

2. Extraction with suitable solvent(s).

3. 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.

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

5. Repeated fractionation of active fractions to isolate pure compound(s).

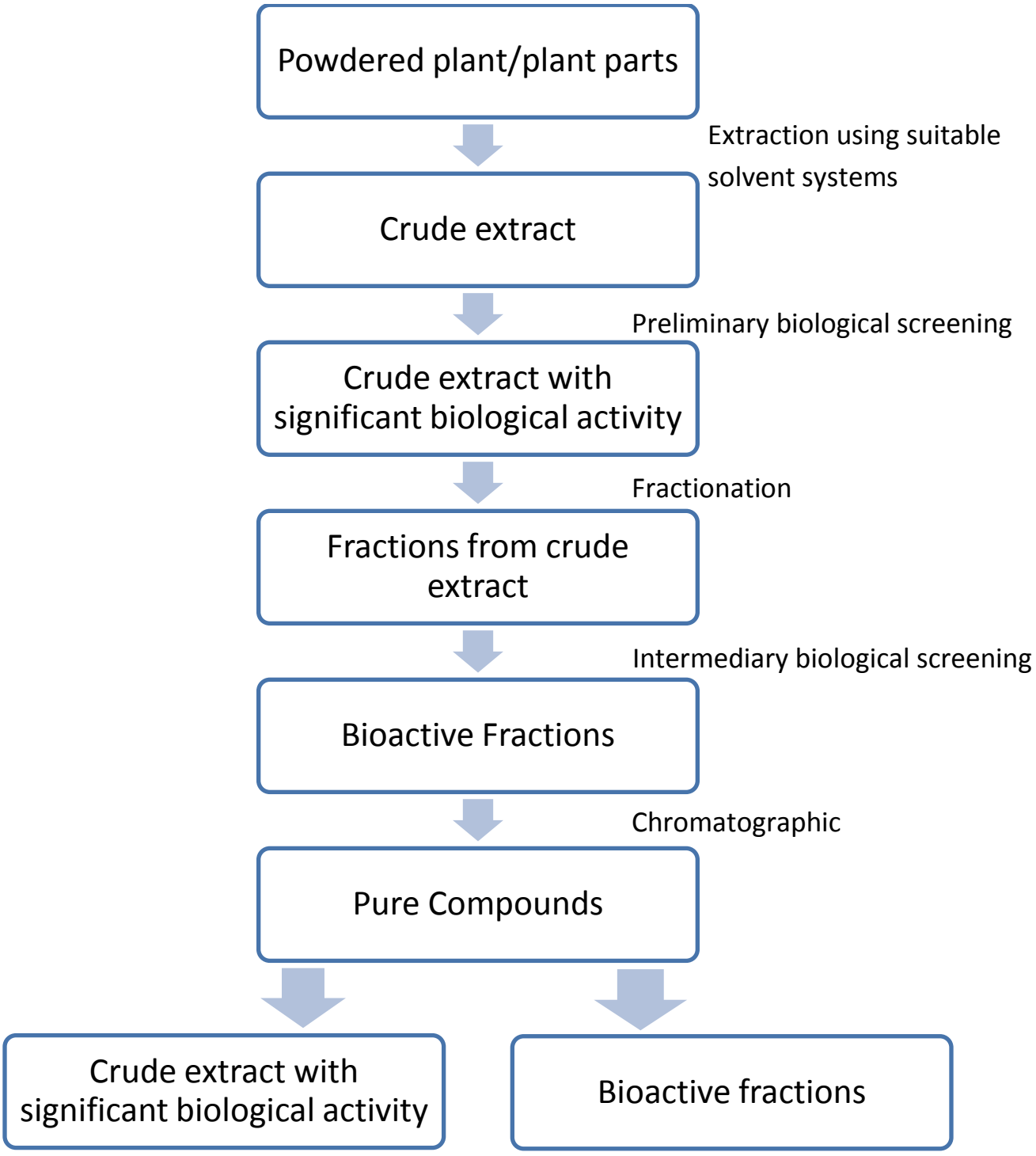
6. Elucidation of chemical structure of pure compound(s) using spectroscopic methods.

7. Evaluation of biological activity of pure compound(s)

8. Toxicological tests with pure compound(s).

9. Production of drug in appropriate dosage forms.

1.4.1 Schematic Diagram of Bioactivity Guided Research of Medicinal plants



1.5 Antioxidant potential of Medicinal Plants

Antioxidants are substances that may protect human cells against the effects of free radicals. Dietary plants contain variable chemical families and amounts of antioxidants. It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants. Studies suggest that a diet high in antioxidants from fruits and vegetables is associated with a lower risk of cancer, cardiovascular disease, Parkinson's disease and Alzheimer's disease. Such diseases have been found to be the result of damage of cells due to free radical generation (Singh et.al., 2013).

1.5.1 Free Radicals and Oxidative Stress

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

When the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system, oxidative stress occurs in cellular system, including the superoxide anion radical, the hydroxyl radical, hydrogen peroxide and the peroxyl are greatly reactive molecules, which consequently generate metabolic products that attack lipids in cel1

membrane or DNA (Halliwell & Gutteridge, 1995). Oxidative stress, involves a series of free radical chain reaction processes, is associated with several types of biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging (Anderson et al., 2000). Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng et al., 1997). Improved antioxidant status helps to minimize the oxidative damage and thus can delay or decrease the risk for developing many chronic age related, free radical induced diseases (Karuna et al., 2009). The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years as the possibility of toxicity of synthetic antioxidants has been criticized (Jayaprakash and Rao, 2000).

Several herbs and herbal formulations are available for the scavenging activity. In addition to this there is a global trend to revive the traditional systems of medicines and renewed interest in the natural remedies for treating human ailments. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases. Almost all organisms are well protected against free radical damage by either enzymes or compounds, such as ascorbic acid, α- tocopherol and gluthione (Singh et.al., 2013).

Phenolic compounds from medicinal plants, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins, possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals. They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers. (Kähkönen et al., 1999; Proestos et al., 2006) There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Hence, the present study was aimed at determining the antioxidant capacities of the plant chosen.

1.5.2 Classification of anti-oxidants

It is of two types (Gupta et al., 2006):

1. Based on solubility:

(a) Hydrophilic antioxidants- They are soluble in water. Water soluble antioxidants react with oxidants react with oxidants in the cell cytoplasm and blood plasma.

(b) Hydrophobic antioxidants- They are soluble in lipids. Lipid soluble antioxidants protect cell membranes from lipid peroxidation.

2. Based on line of defense:

(a) First line defense (preventive antioxidant)-

These are enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GTX), glutathione reductase and some minerals like Se, Mn, Cu etc. SOD mainly acts by quenching of superoxide (O_2), catalase by catalyzing the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. GTX catalyzes the reduction of H_2O_2 and lipid peroxide generated during lipid peroxidation to water using reduced glutathione as substrate.

(b) Second line defense (Radical scavenging antioxidant)-

These are glutathione, Vit C, uric acid, albumin, biliribin, vit E, carotenoids, flavonoid etc. β- carotene is an excellent scavenger of singlet oxygen. Vit C interacts directly with radicals like O_2, OH. GSH is a good scavenger of many free radicals like O_2, OH and various lipid hydroperoxides and may help to detoxify many inhaled oxidizing air pollutants like ozone.

(c) Third line defense (Repair and de-novo enzymes)-

These are a complex group of enzymes for repair of damaged DNA, protein, oxidized lipids and peroxides and also to stop chain propagation of peroxyl lipid radical. These enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane.

1.6 Cytotoxic Screening

Cytotoxicity is the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). To measure the cytotoxicity of a compound derived from plant, a bioassay can be employed in order to provide an estimation of concentration or potency of a substance (drugs, hormones, vitamins, toxins, and antitoxin) by measurement of the biological response that it produces.

Some of the traditional medicine involves the use of crude plant extracts which may contain an extensive diversity of molecules, often with indefinite biological effects. However, most of the available information regarding the medicinal potential of these plants is not provided with credible scientific data. For this reason, several researches have been conducted to determine the toxicity of medicinal plants. A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in plant crude extracts is the Brine Shrimp (*Artemia* sp.) Lethality Assay (BSLA). BSLA is used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes BSLA a very useful bench top method. This assay has been noted as a useful tool for the isolation of bioactive compounds from plant extracts (Olowa and Nuneza, 2013).

In this present study, methanolic extracts of the selected medicinal plant were tested *in vivo* for their cytotoxic effect against the brine shrimp nauplii and relate toxicity results with their known ethno-pharmacological activities.

1.7 Review on *Aglaonema Hookerianum*

1.7.1 Vernacular names

Table 3: Showing the vernacular names of *Aglaonema hookerianum* Schott in different regions (Islam et al., 2012)

Region/Tribal name	*Vernacular names*
Bangla	Habinishak, Patabahar
Chakma	Gach Petic, Shakkosala, Sikkachala
Tanchangya	Shackkatola
Marma	Chekhow, Khaichcha Parabol, Meggey
Tripura	Hatharikhiethok
Khumi	Lykho

1.7.2 Taxonomy

The scientific classification of *Aglaonema hookerianum* Schott is

Kingdom: Plantae

Phylum: Tracheophyta

Class: Liliopsida

Order: Alismatales

Family: Araceae

Genus: Aglaonema

Species: Aglaonema hookerianum

1.7.2 Botanical name/Synonym: *Aglaonema clarkei* Hook.f.

1.7.3 Scientific name: *Aglaonema hookerianum* Schott

1.7.4 Group: Monocot

1.7.5 Plant Family: Araceae

1.7.5.1 The family of Aroids or Araceae

The Araceae, or aroids, is a family of herbaceous monocotyledons with 125 genera and about 3750 species Araceae is a family of monocotyledonous flowering plants in which flowers are borne on a type of inflorescence called a spadix. The spadix is usually accompanied by, and sometimes partially enclosed in, a spathe or leaf-like bract. Also known as the arum family, members are often colloquially known as aroids. The family is predominantly tropical in distribution, with 90% of genera and c. 95% of species restricted to the tropics. Although the greatest number of species originate in South America (including the two largest genera, *Anthurium* and *Philodendron* with over 1500 species between them, the tropics of South East Asia are also very rich, with the large and horticulturally important genera *Alocasia* and *Amorphophallus* (Nicolson, 1969).

The Araceae contains several well-known cultivated foliage and flowering plants. Members of the family are highly diverse in life forms, leaf morphology, and inflorescence characteristics. Life forms range from submerged or free-floating aquatics to terrestrial (sometimes tuberous), and to epiphytic or hemiepiphytic plants or climbers. Leaves range from simple and entire to compound and highly divided, and may be basal or produced from an aerial stem. The family Araceae is defined by bearing small flowers on a fleshy axis (spadix) subtended by a modified leaf (spathe) (Nicolson, 1969).

In some genera the spathe is very conspicuous and brilliantly coloured (e.g., many *Anthurium* species) while in others the spathe is small and leaf-like (e.g., many *Pothosspecies*). In the North American genus *Orontium* the spathe is so reduced that it appears to be absent altogether and in *Gymnostachys*, a peculiar genus restricted to eastern Australia, debate continues as to whether a spathe is in fact present or, indeed, if *Gymnostachys* might be better removed altogether from the aroids. The behaviour of the spathe varies from genus to genus. In some (e.g., *Cryptocoryne*) the spathe completely encloses the spadix, while in others the spathe reflexes to leave the spathe clearly visible (e.g., most *Anthurium*, *Spathiphyllum*). In some genera the spathe is shed as soon as the

inflorescence reaches anthesis, either falling completely (e.g., *Rhaphidophora*) or partially (e.g., *Schismatoglottis*). The spathe ranges in size and shape from 5mm long and simple in *Homalomena humilis* to the fluted and pleated vase 1m wide and 1.5m tall found in *Amorphophallus titanium* (Nicolson, 1969).

The sex of the individual flowers and their arrangement on the spadix are among the characters used to define taxonomic groups. Depending on the genus the spadix may bear either unisexual or bisexual flowers. If bearing bisexual flowers there are uniformly arranged over the spadix. Almost without exception bisexual flowers are subtended by reduced tepals termed a perigon. If unisexual, the flowers are usually arranged with the females at the base of the spadix occasionally terminated by a sterile appendix. In the genus *Arisaema* individual inflorescences are usually either male or female. The sex of the inflorescence in *Arisaema* is governed by the age of the plant, its health, and the type of conditions in which it is growing (Kumar, 2015).

Young plants or mature plants in poor condition or growing in a less than ideal habitat will produce male inflorescences. The ability to alter the sex of the inflorescence in this way is termed paradioecy. Unisexual flowers are almost without exception naked. i.e., lacking a perigon (Nicolson, 1969).

The most recent technical account for the genera is *The Genera of Araceae* (Mayo, Bogner & Boyce, 1997) while a species checklist for the family, *World checklist and bibliography of Araceae* (and Acoraceae) by Govaerts, Frodin etal., appeared in 2002. Aside from floristic accounts and taxonomic treatments the best non-technical account is that of Bown (2000). The classic work on the genus *Arum*, Lords and Ladies (Prime, 1960), is essential reading for anyone wanting to understand the pollination strategy employed by many monoecious aroid taxa (Nicolson, 1969).

All Araceae studied to date display insect pollination. Many, notably *Amorphophallus* have evolved to be pollinated by insects attracted to dung or carrion (saproentomophily). Many tropical species have inflorescences where pollination has evolved in conjunction with bees, wasps and beetles. In species of *Philodendron* investigated to date large dynastid scarab beetles are attracted to the

inflorescences and appear to be the main pollinators (Gottsberger & Amaral, 1984). Many aroids attract pollinators by odour. Inflorescence odours include dung, carrion, rotting fruit, old socks, semen, bad breath, beer, spearmint, cheap sweets and cinnamon (Nicolson, 1969).

Several general have inflorescences that heat up considerably during anthesis, often by as much 20°C above the ambient temperature and often producing at the same time a strong, foul odour. Some genera also offer potential pollinators food in the form of fat bodies (*Dieffenbachia*), sugar solutions (many *Arum* species) or oil droplets (*Amorphophallus*) (Nicolson, 1969).

Within the Aracae, genera such as *Alocasia*, *Arisaema*, *Caladium*, *Colocasia*, *Dieffenbachia*, and *Philodendron* contain calcium oxalate crystals in the form of raphides. When consumed, these may cause edema, vesicle formation and dysphagia accompanied by painful stinging and burning to the mouth and throat, with the symptoms occurring for up to two weeks (Nicolson, 1969).

1.7.6 Macroscopic/Morphological characteristics

It is an annual herb. Stem is erect, 40-50 cm or more, 1.5-2.0 cm thick. Internodes are 1.5-3.0 cm long. Petioles are 14-24 cm long, 0.7-0.9 (1.2) times as long as the leaf-blade. Sheaths membranous, (4) 8-15 cm long, (0.2) 0.5-0.6 (0.8) times as long as the petiole. Leaf-blade ovate to elliptic or lanceolate to narrowly elliptic, 20-27 cm long (5.3) 7-12 cm wide, length/width ratio 1 : 2.0-2.9 (3.8) ; base often unequal, rounded, obtuse or broadly acute, rarely acute; apex often apiculate, acuminate to gradually or suddenly long acuminate (acumen to 2.1 cm long from point of 1 cm blade width to apex) ; variegation none; venation weakly to strongly differentiated into 7-13 primary lateral veins which diverge from the midrib at (30') 40"-50". Peduncles 1-3 together, 10-21 cm long. Spathe 3.7-6.0 cm long, decurrent for (0.6) 1-1.5 (2) cm. Stipe none. Spadix thin-cylindric, 2.5-4.0 cm long, equaling to 0.8 cm short of spathe apex; pistillate portion 0.3-0.6 cm long, attached to spathe, pistils ca. 10-15; staminate portion 2.0-3.7 cm long, 0.3-0.6 cm thick. Fruits red, large when ripe, (1.7) 2-3 cm long, (0.7) 0.9-1.4 cm thick (Nicolson, 1969).

1.7.7 Distribution

Darjiling, Assam, Bhutan, and Myanmar. In Bangladesh, it is found in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. (Motaleb, 2013)

1.7.8 Distinguishing Features

The distinctive characteristics of *Agalonema hookerianum* are (Nicolson, 1969)-

- ✓ sessile spadix,
- ✓ spadix equaling the spathe
- ✓ large fruits,
- ✓ a long peduncle (compared with *A. ovatum* and *A. modestum),*
- ✓ restriction to the above mentioned location or distribution

1.7.9 Habitat

Below 1000 m, in deep shade of forest receiving more than 80 inches of annual rainfall (Nicolson, 1969).

1.7.10 Flowering & Fruiting time

June-July, probably influenced by the onset of the summer monsoon. Fruit berry, bright red (Nicolson, 1969).

1.7.11 Propagation: Propagated by rhizomes (Nicolson, 1969).

1.7.12 Chemical constituents: Not known (Nicolson, 1969).

(a)

(b)

(c)

(d)

Figure 1: *Aglaonema hookerianum* Schott (a) Fruit, (b) Leaves, (c) Stem, (d) Whole Plant

1.7.13 Local Uses

Different parts of this plant are applicable for various treatments-

Table 4. Local uses of *Aglaonema hookerianum* Schott in different regions of Bangladesh (Biozid et al., 2015; Rahman et. al., 2007; Motaleb, 2013)

Plant part	**Tribe**	**Local use**
root	Chakma	The sap of the root is taken orally for the treatment of conjunctivitis and constipation
leaf	Tanchangya	A leaf extract is taken and a paste of the leaves is applied to the whole body for the treatment of hysteria people in Bangladesh
Spathe	Khumi, Marma and Tripura	Extracted spathe juice (two table spoon) taken orally

		twice a day for 2-3 days for stomachache.

1.7.14 Medicinal uses

- The species is used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus (Uddin and Rahman, 2006).

1.7.15 Other uses

It is also used as vegetable. Sometimes it has been used as ornamental plants (Motaleb, 2013).

1.7.16 Toxicology

- **Oxalates:** The juice or sap of the plant contains oxalate crystals. These needle-shaped crystals can irritate the mucous membranes in skin, mouth, tongue, and throat, resulting in throat swelling, breathing difficulties, burning pain, and stomach upset (Islam et al., 2012).
- **Dermatitis:** The juice of the plant may cause a skin rash or irritation (Islam et al., 2012).

Table 5. Other species under Aglaonema

Species	
Aglaonema brevispathum	*Aglaonema ovatum*
Aglaonema chermsiriwattanae	*Aglaonema philippinense*
Aglaonema commutatum	*Aglaonema pictum*
Aglaonema cochinchense	*Aglaonema pumilum*
Aglaonema cochinchinense	*Aglaonema rotundum*
Aglaonema cordifolium	*Aglaonema tenuipes*

Aglaonema costatum	*Aglaonema tricolor*
Aglaonema crispum	*Aglaonema vittatum*
Aglaonema modestum	*Aglaonema nebulosum*
Aglaonema nitidum.	*Aglaonema marantifolium*
Aglaonema oblongifolia	*Aglaonema flemingianum*
Aglaonema simplex	*Aglaonema densinervium*

Chapter Two

LITERATURE REVIEW

2.1 Literature Review

2.1.1 Phytochemical screening, cytotoxicity and antibacterial activities of two Bangladeshi medicinal plants

The ethanolic extracts of leaves of *Aglaonema hookerianum* Schott (Family: Araceae) were investigated for the phytochemical screening and assaying cytotoxicity and antibacterial activities. The brine shrimp lethality bioassay of ethanolic extracts of *Aglaonema hookerianum* revealed cytotoxic activities with LC_{50} 5.25 (microg mL^{-1}) and LC_{90} 9.55 (microg mL^{-1}). Antibacterial activities of the extract were examined against some gram positive bacteria such as Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus, also gram negative strains of Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Salmonella typhi, Salmonella paratyphi and Vibrio cholerae. Using Agar disc diffusion method, antibacterial efficacy of the extract (500 microg $disc^{-1}$) was observed and compared with the zones of inhibition of Amoxicillin at concentration of 10 microg $disc^{-1}$. The extract produced significant antibacterial activity which indicates a useful source for the development of new potent antibacterial agents (Roy et al., 2011).

2.1.2 *In-vitro* anti-atherothrombosis activity of four Bangladeshi plants

Another study was conducted to investigate the thrombolytic activity of ethanol extracts of *Aglaonema hookerianum*. The study was carried out in-vitro with streptokinase being the reference standard and ethanol as negative control. The extract produced 11.18% of clot lysis with reference to streptokinase (81.08%) (Islam et al., 2012).

2.1.3 A comparative study of thrombolytic effects of methanolic extract of Bridelia stipularis and Aglaonema hookerianum leaf

Crude methanol extract of *B. stipularis* and *A. hookerianum* leaf was evaluated to compare the thrombolytic activities among them using the in vitro clot lysis model. Venous blood taken from five healthy workers was allowed to form clots. Then the clot was weighed and treated with the test samples from the plant extracts to disrupt the clots. The percentage of clot lysis determined from the weight of clot before and after treatment was compared with the streptokinase as the positive control and water as the negative control. Form the study, it was observed that *B. stipularis* and *A. hookerianum* showed 33.42 ± 3.37% and 24.72 ± 2.75% of clot lysis respectively. As the result was compared with the reference drug streptokinase (63.54 ± 2.61%), it was found that *A. hookerianum*

showed significant ($p < 0.001$) percentage of clot lysis but comparably less significant than the two plants (Biozid et. al., 2015).

2.1.4 Shuktani – a new ethno-medico recipe among the Sylheti Bengali Community of Barak valley, Southern Assam, India

A survey was performed on the ethnobotanical study of Barak valley of Southern Assam for 10 years during which many new traditional prescriptions and recipes were known. Among such recipes Shuktani is one of the traditional and common recipes that is still in regular practice amongst the community of Barak valley of Sothern Assam. The preparation is being used for various diseases including stomach disorders and like diarrhoea, dysentery, indigestions, etc. and as a recipe for women as post parturition treatment, weakness and lactation. The recipe of Shuktani is prepared with the uses of 35 species of Angiosperms consisting of the leaves of 23, vegetative buds, fruits and seeds of 4 each, stems and flowers of 2 each of the plants in either liquid form or in powdered form which comprises *Aglaonema hookerianum* as one of the marked component (Nath and G Maiti, 2012).

2.1.5 Polyhydroxyalkaloids in the Aroid Tribes Nephthytideae and Aglaonemateae: Phytochemical Support for an Intertribal relationship

In another survey of polyhydroxyalkaloids, living and herbarium material was used in species of 52 genera of Araceae which revealed the existence 2,5-dihydroxymethyl-3,4 dihydroxypyrrolidine (DMDP) and α-homonojirimycin (HNJ) in leaves of Aglaonema Schott. Levels were high in living plants, ranging from 0.1 to 1% dry weight DMDP and 0.04 to 0.6% HNJ. α-3,4-di-epihomonojirimycin, an isomer of HNJ, were also present (Kite et al., 1997).

2.1.6 Antihyperglycemic Effects of N-Containing Sugars from *Xanthocercis zambesiaca, Morus bombycis, Aglaonema treubii,* and *Castanospermum australe* in Streptozotocin-Diabetic Mice

This study evaluated eight structurally related nitrogen-containing sugars, fagomine (1), 4-*O*-β-d-glucopyranosylfagomine (2), 3-*O*-β-d-glucopyranosylfagomine (3), 3-epifagomine (4), 2,5-dideoxy-2,5-imino-d-mannitol (5), castanospermine (6), α-homonojirimycin (7), and 1-deoxynojirimycin (8) present in *Morus bombycis*, *Aglaonema treubii*, and *Castanospermum australe* for antihyperglycemic effects in streptozotocin (STZ)-diabetic mice. Compounds 1, 2, 5, and 6 reduced the blood glucose level after intraperitoneal injection of 150 μmol/kg. Due to compound 1 there was

increased plasma insulin level in STZ-diabetic mice and potentiated the 8.3-mM glucose-induced insulin release from the rat isolated-perfused pancreas. Antihyperglycemic action may be partly contributed to the fagomine-induced stimulation of insulin release (Nojima et al., 1998).

2.1.7 Interior plants for sustainable facility ecology and workplace productivity

In this work, Burchett et al. examined the capacity of three plants including Aglaonema modestum for cleaning the environment from benzene, toluene, xylene and n-hexane, which are used as industrial solvents for furnishings. They showed that the contaminants concentrations decreased gradually below the detection limits of the gas chromatograph (Mosaddegh et al., 2014).

2.1.8 Homonojirimycin Isomers and Glycosides from *Aglaonema treubii*

From a study performed on a 50% aqueous ethanol extract of *Aglaonema treubii* found to be potent in inhibiting α-glucosidase, several compounds were revealed when treated to various ion-exchange column chromatographic steps. The compounds were 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (1), α-homonojirimycin (2), β-homonojirimycin (3), α-homomannojirimycin (4), β-homomannojirimycin (5), α-3,4-di-*epi*-homonojirimycin (6), 7-*O*-β-d-glucopyranosyl-α-homonojirimycin (7), and 5-*O*-α-d-galactopyranosyl-α-homonojirimycin (8). Compounds 1 and 2 are known inhibitors of various α-glucosidases. Compounds 6 and 8 are new natural products.
Compounds 3−5 and 7 have been chemically synthesized previously, but this report is known to be the first incidence of their natural occurrence (Asano et al., 1997).

2.1.9 Photocytotoxic pheophorbide-related compounds from *Aglaonema simplex*

In a screening program evaluated on the leaves and stems of Aglaonema simplex for new photosensitizers, five pheophorbide-related compounds were found. Compounds 1-3 and 5 are pheophorbide and hydroxy pheophorbide derivatives of chlorophyll a and b as shown by the detailed spectroscopic analyses. Compound 4 was isolated for the first time from the Araceae family which was identified as 15(1)-hydroxypurpurin-7-lactone ethyl methyl diester. An MTT-based short-term survival assay was applied on all five compounds which showed that they exhibited moderate-to-strong photocytotoxic activities towards human leukemia (HL60) and two oral squamous carcinoma cell lines

(HSC-2 and HSC-3). Compounds 4 and 5 displayed the strongest photocytotoxicities, with IC(50) values of 0.30-0.41 muM. Compounds 1-3 with ethly chains at C(17(3)) were less photocytotoxic than the parent pheophorbide a (5) (Chee et al., 2005).

Rationale of the study

Aglaonema hookerianum is a medicinal plant distributed in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. The species is traditionally used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus and in stomachache, conjunctivitis and constipation by various communities of the region. The plant proved to be effective for the treatment of various diseases and so it is profoundly used for its medicinal value. This establishes *Aglaonema hookerianum* a potential study material for both phytochemical and pharmacological investigations. So experimental studies were carried out to evaluate different pharmacological activities of the methanol extract of *Aglaonema hookerianum*.

Aim of the Present Study

New drug discovery is facing serious challenges due to reduction in number of new drug approvals coupled with exorbitant rising cost. Advent of combinatorial chemistry provided new hope of higher success rates of new chemical entities; however, even this scientific development has failed to improve the success rate in new drug discovery. This scenario has prompted us to come out with a novel approach of integrated drug discovery, where Ayurvedic wisdom can synergize with drug discovery from plant sources. Initial steps in new drug discovery involve identification of new chemical entities, which can be either sourced through chemical synthesis or can be isolated from natural products through biological activity guided fractionation. The sources of many of the new drugs and active ingredients of medicines are derived from natural products. The starting point for plant-based new drug discovery should be identification of the

right candidate plants by applying Ayurvedic wisdom, traditional documented use, tribal non-documented use, and exhaustive literature search.

Aglaonema hookerianum is a rare medicinal plant found in Bangaldesh and is used traditionally for many years by local communities of Chittagong Hill Tracts and Sylhet for various ailments. After vigorous investigation for the search of literature reports it was found that only few activities has been performed on the plant to evaluate its medicinal value. Very little is known about its constituents that are responsible for its pharmacological studies. So, taking this into account, several studies need to be performed to investigate the traditional uses of this plant.

The principal aim of the present study is to perform an experiment based on a sound scientific background which supports the traditional uses of the plant. The methanolic extract of *Aglaonema hookerianum* is used to-

- To conduct a cytotoxic investigation by brine shrimp lethality bioassay.
- To evaluate the antioxidant activity by reducing power method.

Chapter Three

MATERIALS AND METHODS

3.1 Preparation of plant extract for experiments

3.1.1 Collection

Aglaonema hookerianum was collected from Chittagong hill tract area. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen (Accession No. 40633) has been deposited for future reference.

3.1.2 Preparation of Plant material

At first the plants were cleaned to remove dust, soil etc. from them. After this the whole amount of plant was dried in the oven at considerably low temperature. The dried plants were ground into coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. The amount of powder was 550g. During powdering of sample, the blender was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blender.

3.1.3 Extraction

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

3.1.4 Filtration

After the extraction process the plant extract was filtered with sterilized cotton filter followed by Whatman No.1 filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a conical flask and covered with aluminium foil paper for later treatment with rotary evaporation.

3.1.5 Evaporation and extract preparation

For evaporating the solvent and collect for reuse Heidolph rotary evaporator machine was used featuring a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. The evaporation was done at 55 degree Celsius temperature. The number of rotation per minute was selected at 110 RPM. The pressure of the vacuum pumper machine (Biometra) was 6 bar. The water flow through the distillation chamber was also provided in a satisfactory flow rate.

The solvent that was evaporated flowed along the condenser where it was condensed back to liquid form. 70% of the solvent was collected and could be reused. The plant extract was collected from the evaporating flask and the solvent is collected from the receiving flask. The extract is transferred into a 50 ml beaker and covered with aluminum foil. With a sharp utensil, the foil was penetrated to produce many pores so that it could be air dried into a concentrated solid residue.

Figure 2: Rotary evaporator

3.2 Antioxidant Assay (Reducing Power) of *Aglaonema hookerianum*

3.2.1 Principle

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700nm. Higher absorbance of the reaction mixture indicates higher reductive potential (Jayanthi and Lalitha, 2011).

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

3.2.2 Materials and Equipment

- Test tubes
- UV-2450 spectrophotometer
- Test tube holder.
- Beaker
- Electronic balance

3.2.3 Reagents and Chemicals

- Methanol
- Potassium ferricyanide
- Trichloro Acetic acid
- Ferric Chloride ($FeCl_3$)
- Ascorbic acid as standard
- Distilled water

3.2.4 Experimental procedure

The reducing power of extracts from *Aglaonema hookerianum* was determined according to the method of Oyaizu (1986). The method follows the steps below-

1. 2.0 ml of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes.

2. 2.5 ml of Potassium ferricyanide [$K_3Fe(CN)_6$] 1% solution was added into the test tubes.

3. Then the test tubes were incubated for 10 minutes at 50 ^{0}C to complete reaction.

4. 2.5 ml of trichloro acetic acid (10%) was added into the test tubes.

5. The total mixture was centrifuged at 3000 rpm for 10 minutes.

6. 2.5 ml supernatant solution was withdrawn from the mixture and mixed with 2.5 ml of distilled water.

7. 0.5 ml of ferric chloride (0.1%) solution was added.

8. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer (Shimadzu UV PC-1600) against blank.

9. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the sample solution.

10. The absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation was also taken.

3.3 Cytotoxicity Assay of *Aglaonema hookerianum*

3.3.1 Principle

The in vivo lethality in a simple zoological organism, such as the brine shrimp lethality test (BST) might be used as a simple tool to guide screening and fractionation of physiologically active plant extracts, where one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. This general bioassay detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply pharmacology at a higher dose, thus if we find toxic compounds, a lower, non-toxic, dose might elicit a useful, pharmacological, perturbation on a physiologic system. However, it has been demonstrated that BST correlates reasonably well with cytotoxic and other biological properties. Thus BST provides a method of screening the plant fractions for many new useful activities that were unknown to the world rather than continuing search for specific activities having the chance of not being present (Kesavan et al., 2011).

Brine shrimp eggs are hatched in simulated sea water to get live nauplii. By the addition of calculated amount of Dimethyl sulfoxide (DMSO), desired concentrations of the test sample is prepared. The nauplii are counted by visual inspection and are taken in test tubes containing 5 ml of simulated sea water. Then samples of different concentrations are added to pre-marked test tubes using micropipettes. Then the test tubes are left for 24 hours. Survivors are counted after 24 hours.

3.3.2 Materials and reagents required

- *Artemia salina* leach (brine shrimp eggs)
- Sea salt (NaCl)
- Dimethyl sulfoxide (DMSO)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes, Micropipette
- Glass vials
- Test samples of experimental plants
- Magnifying glass

3.3.3 Procedure

For Cytotoxicity study, Brine shrimp lethality bioassay method was applied for the evaluation of cytotoxic property of methanolic crude extracts of the leaves of *Aglaonema hookerianum*, to calculate the Lethal Concentration at 50% mortality (LC_{50}), the method of which was modified from the original procedure of utilizing the live nauplii of *Artemia salina* by Meyer, et al. (1982). The steps are as followed –

3.3.4 Preparation of Sea Water

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

3.3.5 Hatching of Brine Shrimp

At first, 38gm Iodine – free Sodium chloride (NaCl) salt was dissolved into 1L Distilled Water in a transparent glass jar to prepare isotonic solution of NaCl (3.8% NaCl Solution) for simulating the Sea Water in which the *Artemia salina* cysts hatches. The underlying reason for using Iodine-free NaCl was that the Iodine itself has cytotoxic activity which can modify the actual result. Then sufficient amount of *Artemia salina* cysts was poured in the solution.

The solution was then left for 24 hrs for incubation under sufficient light and air flow. After 24 hrs of incubation tiny pink or white colored nauplii were visible in stagnant water by turning off the air flow which will attract the nauplii towards the light.

3.3.6 Preparation of Test Solutions

Clean and dry test tubes were taken. These test tubes were used for ten different concentrations (one test tube for each concentration) of test samples and another one test tube for control test. Each of the test tubes was pre-marked at 5mL.

3.3.7 Preparation of the Test Samples of Experimental Plant

Then, 4mg of methanol crude extract of *Aglaonema hookerianum* was weighed and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions.

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

By using a Pasteur Pipette, 10 nauplii were transferred to each of the test tubes of varying concentration. To facilitate the counting of the nauplii, the stem of the pipette can be held against a lighted background. The test tubes were maintained under illumination.

3.3.8 Preparation of the Negative Control Group

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

3.3.9 Counting Of Nauplii

After 24 hours, the number of survivors in each test tube was counted with the aid of a magnifying glass and the percent (%) deaths at each concentration were determined. The lethal Concentration at 50% Mortality was calculated by using Linear Regression Analysis in Microsoft Excel 2010.

$$\textbf{Percentage (\%) Mortality} = \frac{\textbf{No of Shrimp Died}}{\textbf{Total No of Shrimp in Each Test}} \times \textbf{100}$$

Chapter Four

RESULTS AND DISCUSSIONS

4.1 Results and Discussions

4.1.1 Results of Cytotoxicity Assay of *Aglaonema hookerianum*

After 24hrs, the test tubes were inspected using a magnifying glass and the number of survivors counted. The results of brine shrimp lethality bioassay are provided in the table. Test samples showed different mortality rate at different concentration. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a median Lethal Concentration (LC_{50}) value. This represents the concentration of the methanolic 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. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

$$\text{Percentage (\%) Mortality} = \frac{\text{No of Shrimp Died}}{\text{Total No of Shrimp in Each Test}} \times 100$$

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.

4.1.2 Preparation of Standard Curve

Table 6: Results for Cytotoxic assay of Tamoxifen (standard) on shrimp nauplii.

Concentration(C) (µg/ml)	Log C	No. of nauplii taken	No. of nauplii dead	% mortality	Value of x (log LC_{50})	LC_{50} (µg/ml)
400	2.60206	10	10	100		
200	2.30103	10	9	100		
100	2.00000	10	8	80		
50	1.69897	10	7	70		

25	1.39794	10	5	70	0.989	13.38
12.5	1.09691	10	5	50		
6.25	0.79588	10	4	40		
3.125	0.49485	10	3	40		
1.5625	0.19382	10	2	30		
0.78125	-0.10721	10	1	10		

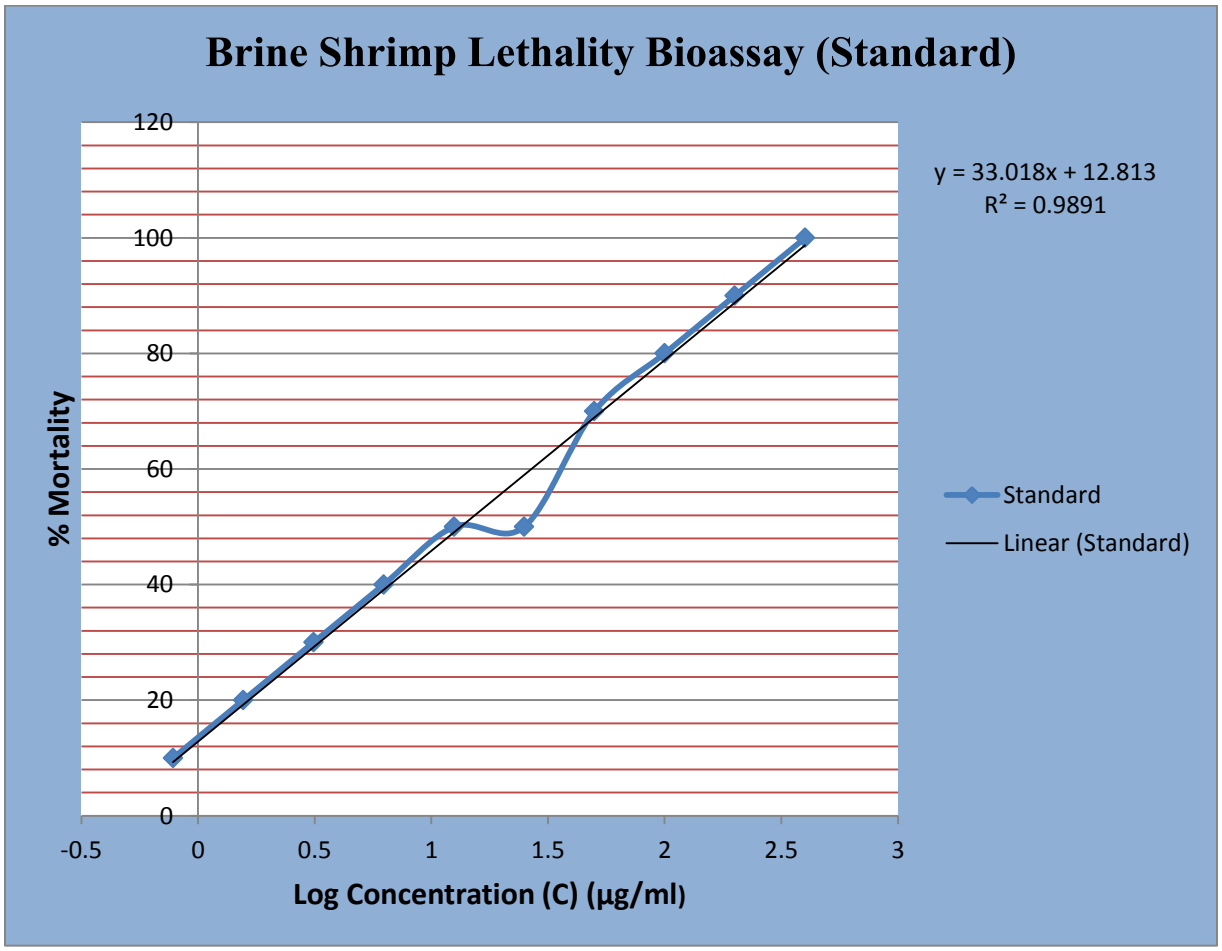


Figure 3: Effects of various concentrations of Tamoxifen (standard) on the viability of brine shrimp nauplii after 24 hrs of incubation.

4.1.3 Preparation of Methanol Fraction Curve

Table 7: Results for Cytotoxic assay of *Aglaonema hookerianum* (methanol extract) on shrimp nauplii.

Concentration(C) (µg/ml)	Log C	No. of nauplii taken	No. of nauplii dead	% mortality	Value of x (log LC_{50})	LC_{50} (µg/ml)
400	2.60206	10	8	80	1.652	44.8
200	2.30103	10	6	60		
100	2.00000	10	6	60		
50	1.69897	10	5	50		
25	1.39794	10	4	40		
12.5	1.09691	10	4	40		
6.25	0.79588	10	4	40		
3.125	0.49485	10	1	10		
1.5625	0.19382	10	1	10		
0.78125	-0.10721	10	1	0		

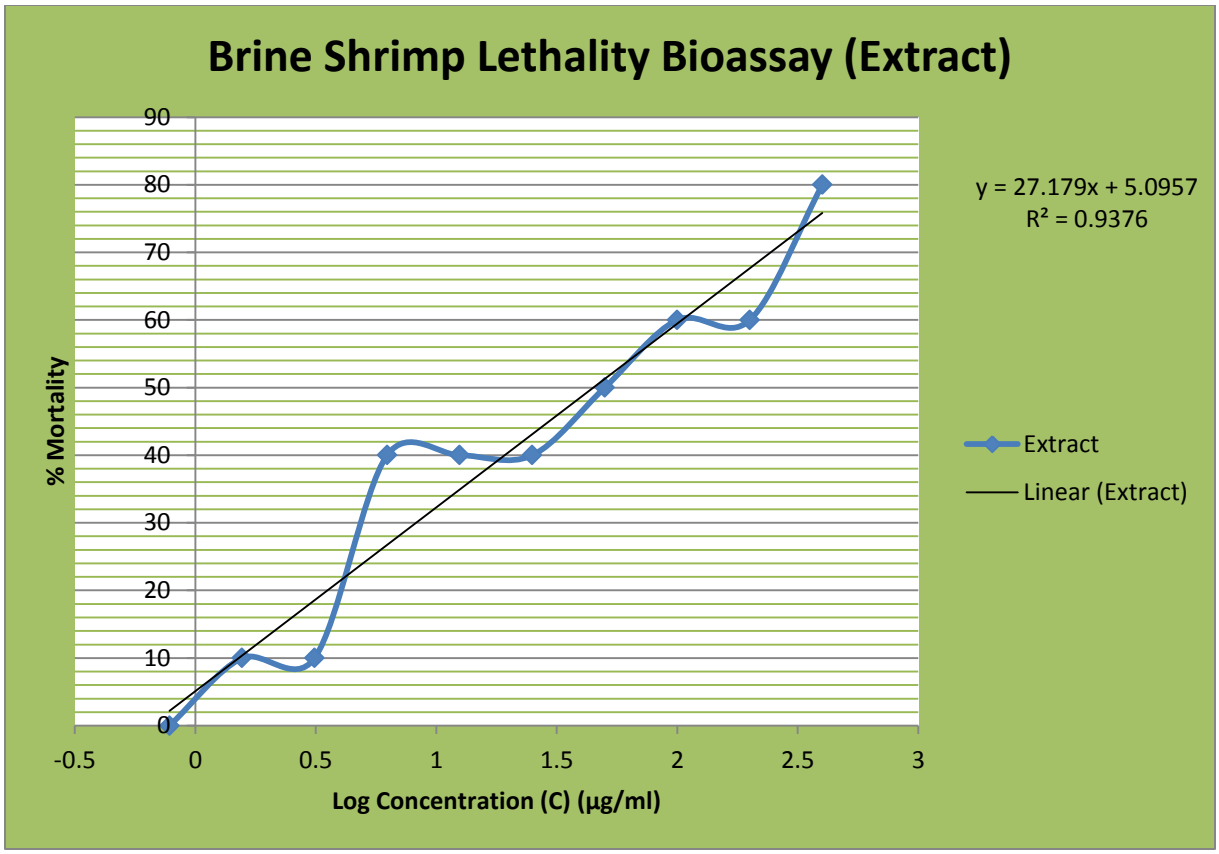


Figure 4: Effects of various concentrations of methanol extract of *Aglaonema hookerianum* on the viability of brine shrimp nauplii after 24 hrs of incubation.

From the investigation of in vivo lethality of brine shrimp, it was observed that when the shrimps was exposed to different concentrations of the samples, there was varying degree of lethality. The regression analysis produced a linear correlation between the mortality rate and the concentration of samples in both case of Tamoxifen and methanol extract. It is evident from the data, the percent mortality of brine shrimp nauplii was found to increase gradually with the increase in the concentration of the test samples. Maximum mortalities occurred at the highest concentration of 400µg/ml, whereas the least mortalities occurred at concentration 0.78125µg/ml as shown in **Table 6** and **Table 7**.

Table 8: Cytotoxic activity of Tamoxifen and methanol extract of *Aglaonema hookerianum*

Sample	**Linear regression equation**	**R^2 value**	**LC_{50} (µg/ml) after 24hr**
Tamoxifen (Standard)	y = 33.021x + 12.806	0.989	13.38
Methanol Extract	y = 32.414x + 18.566	0.938	44.8

From this experiment, it is observed that Tamoxifen (Standard) and methanolic extract of *Aglaonema hookerianum* possess cytotoxic activities having LC_{50} values of 13.38 µg/ml and 44.8 µg/ml respectively as shown in the **Table 8**. R^2 value for methanolic fraction is 0.938 whereas for Tamoxifen (Standard) is 0.989 which is less than the standard and it shows that the fraction is less potent in exhibiting cytotoxic activity against brine shrimp nauplii.

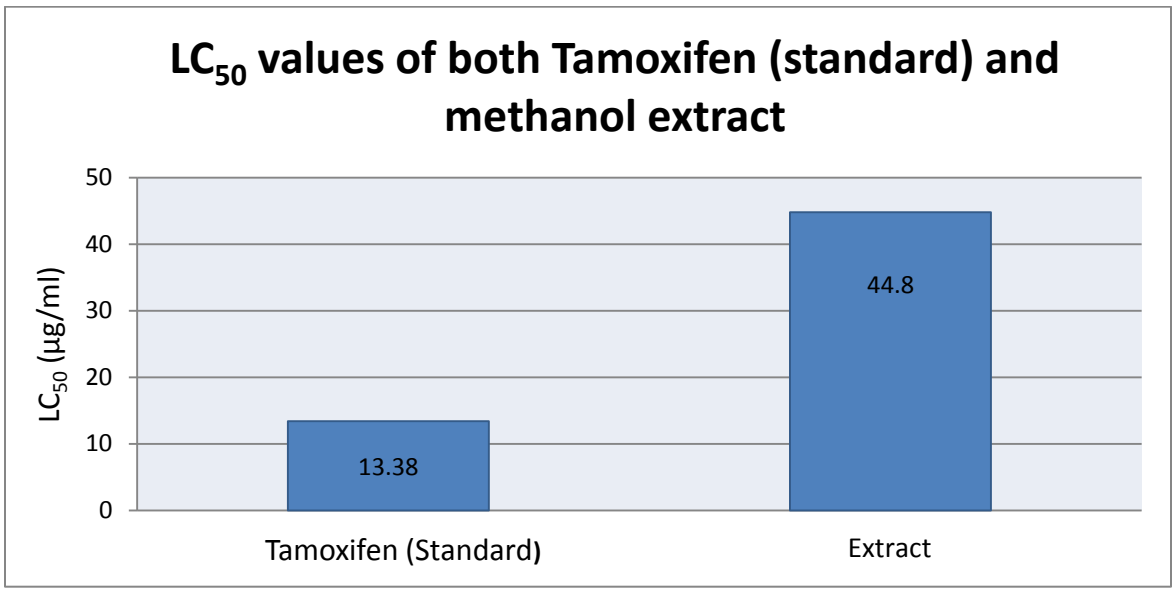


Figure 5: Comparison between LC_{50} valus of Tamoxifen and extract

The figure vividly illustrates the lethal concentration required to kill 50% of the sample population (LC_{50} value) by both the Tamoxifen (Standard) and methanolic extract of *Aglaonema hookerianum*. The higher LC_{50} value of plant extract indicates that it requires higher concentration than standard to reach its lethal dose and it is therefore associated to be less potent than the standard.

Meyer et al. (1982) reported that, if the brine shrimp lethality assay displayed LC_{50} of <1000 µg/ml of natural derived products was known to contain physiologically active principles. The methanolic extract of *Aglaonema hookerianum* studied in this work

showed significant lethality against brine shrimp with LC_{50} value <1000 µg/ml, which has been successfully used as a simple biological test to guide the fractionation process of biological extracts in order to detect active pharmacological compounds.

4.2.1 Results of Antioxidant Assay of *Aglaonema hookerianum*

The methanolic extract of *Aglaonema hookerianum* was evaluated for the reducing power assay based on the principle of increase in the absorbance of the reaction mixtures. The results of antioxidant activity of the extract and standard are depicted in **Table 8**. The results of absorbance and concentration are plotted for the extract and the standard in the **Figure 6**. In this assay, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples and hence increase in antioxidant activity. Here, Ascorbic acid at various concentrations was used as standard.

4.2.1 Preparation of Curve of the Extract

Table 9: Data Table for the Antioxidant Assay of *Aglaonema hookerianum*

Concentration (µg/ml)	Absorbance
200	0.479
150	0.328
100	0.311
50	0.196

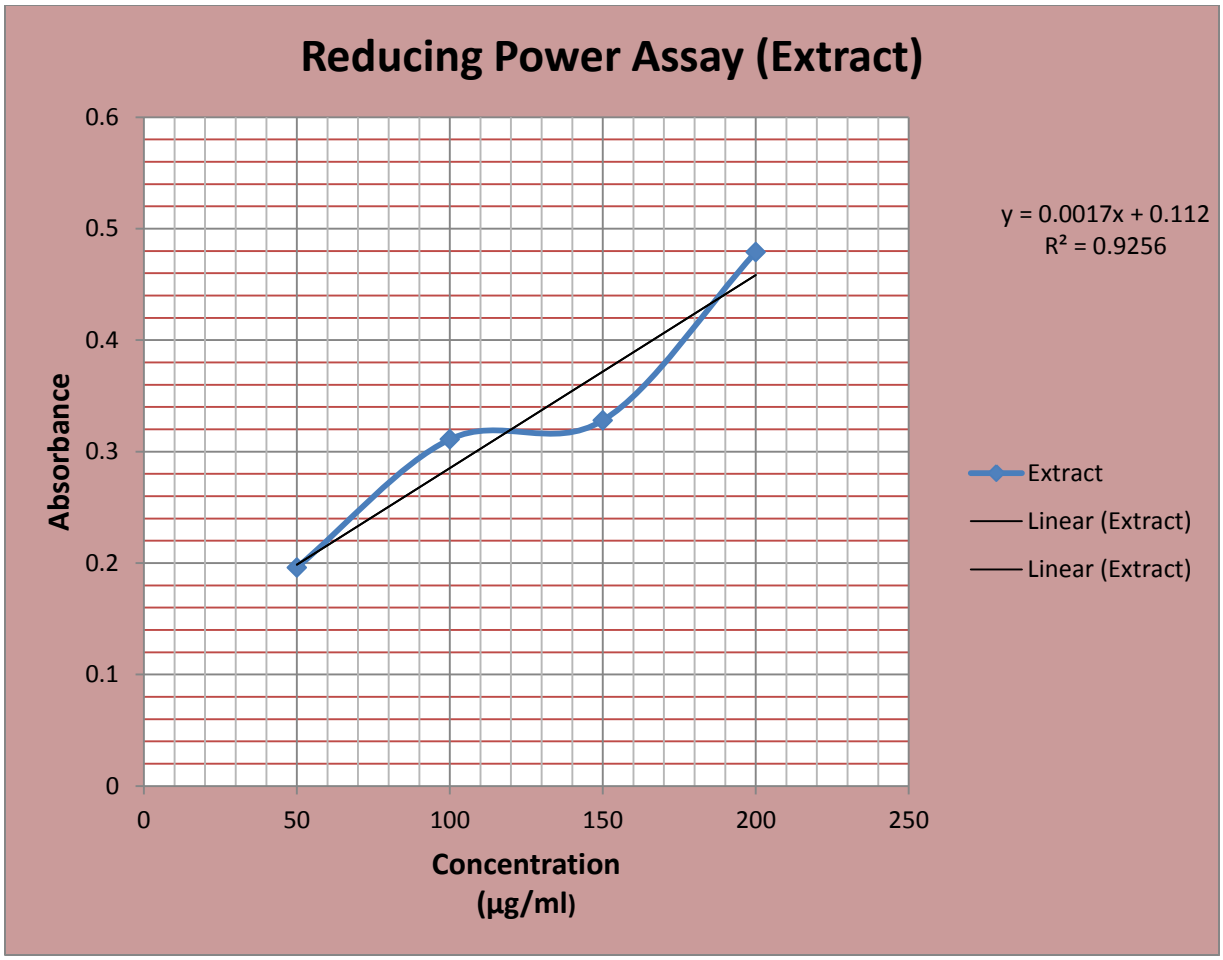


Figure 6: Reducing Power Activity of extract *Aglaonema hookerianum*

4.2.2 Preparation of Standard Curve

Table 10: Data Table for the Antioxidant Assay of Ascorbic Acid

Concentration (µg/ml)	Absorbance
200	0.489
150	0.386
100	0.290
50	0.237

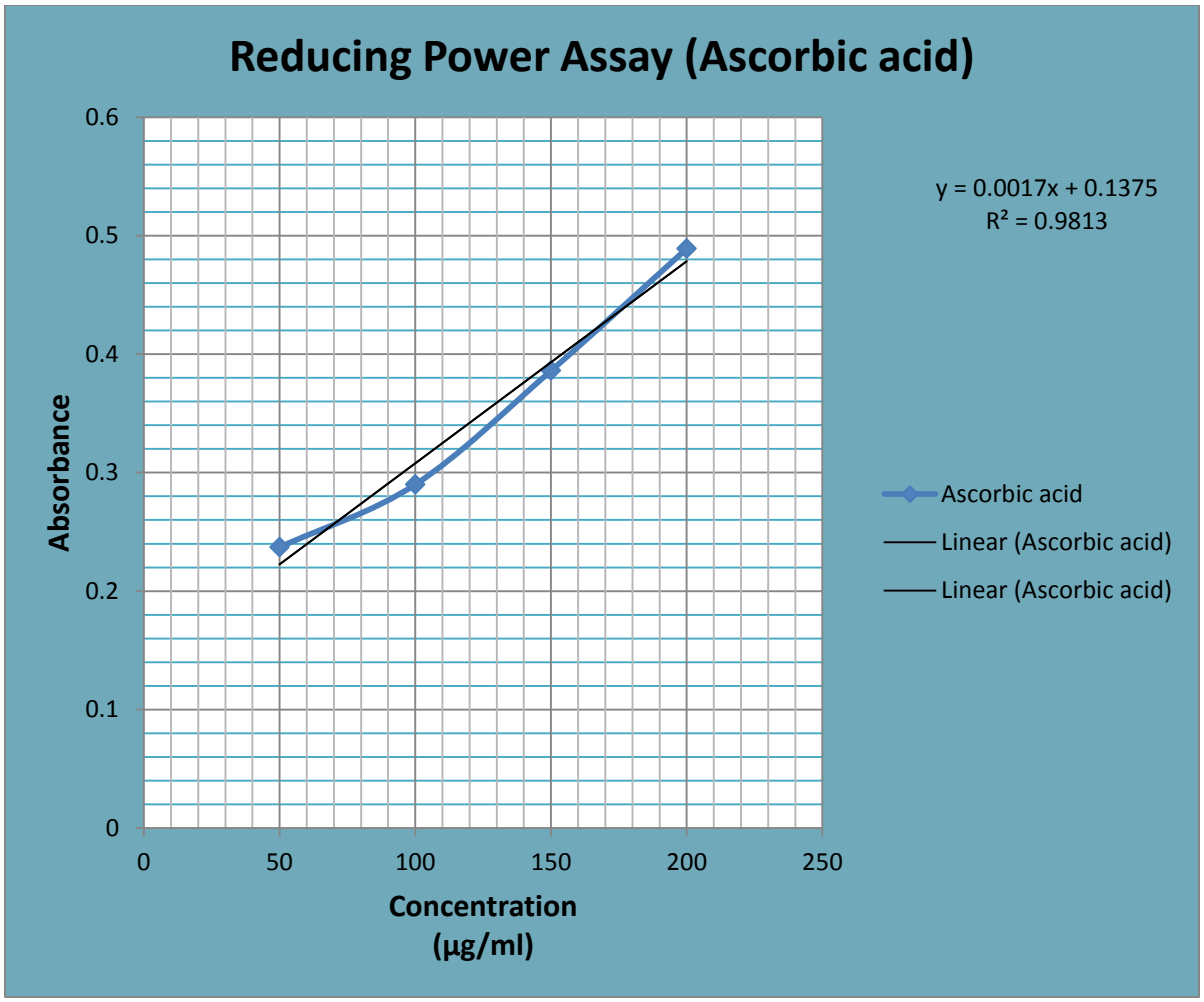


Figure 7: Reducing Power Activity of extract Ascorbic Acid

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.

The reducing power assay reveals that the extract showed significant antioxidant activity which is due to the presence of reducing compounds in the extract.

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. There is a proportionate increase in absorbance as the concentration of the extract and the standard increases. So the higher absorbance of methanol extract may be due to its strong reducing power potential.

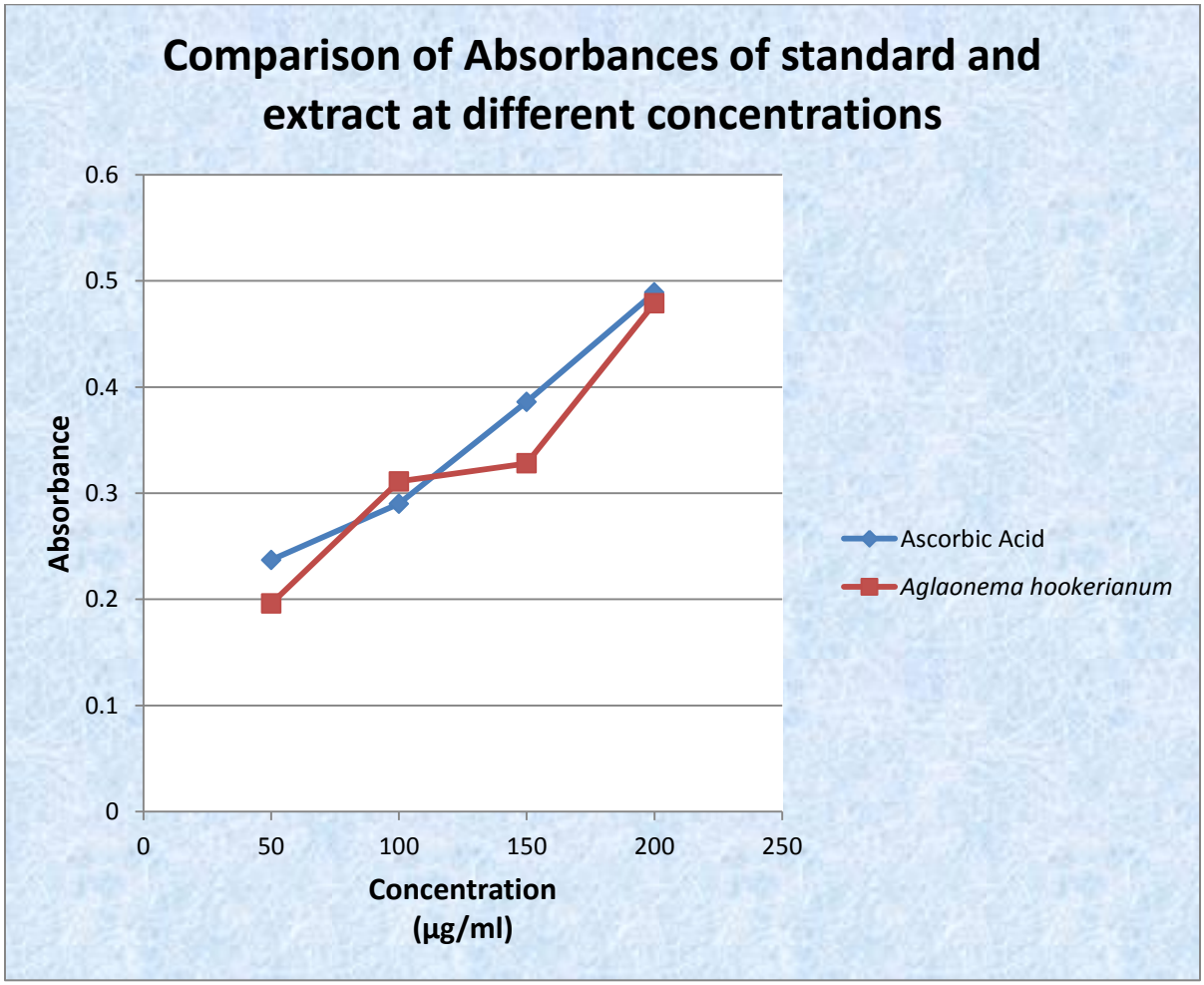


Figure 8: Comparison of the reducing ability of standard and extract over different concentrations

The **Figure 8** shows a linear relationship exists between the concentration and absorbance for both the extract and the standard samples. As concentration increases, the absorbance increases as well. Compared to the standard, the increase in reducing power

of the extract is slightly less and the absorbance within a given range of concentration does not increase sharply, which suggests there are active compounds which possess antioxidant activity but are not potent at the required concentration.

Many researchers have reported that, the reducing power is generally associated with the reductones and might be due to hydrogen-donating ability. The reducing power of the extract of *Aglaonema hookerianum* might contain reductone, which could stabilise free radicals and terminate radical chain reactions and this might be due to the hydrogen-donating abilities (Sarikurkcu, 2011). Therefore, the antioxidant activity of the extracts may be related to their reducing power.

Chapter Five

CONCLUSION

5.1 Conclusion

The results of my study clearly establish the fact that the methanol extract of *Aglaonema hookerianum* possesses both cytotoxic and antioxidant activity.

The crude methanolic extract of *Aglaonema hookerianum* was evaluated for the screening of antioxidant activity by using reducing power assay method. It can be concluded from the results of my in vitro study that the plant extract contained substances with reducing potential as it was able to reduce potassium ferriccyanide to potassium ferrocyanide which subsequently gives a colored complex after reaction with ferric chloride. The reducing power of the extracts can be associated to the presence of biologically active compounds in the extract and literature reports are evident that the reducing power of bioactive compounds is associated with antioxidant activity thus a relation is evidenced between reducing power and the antioxidant effect. Thus the information serves as a potential indicator for the possibility of being a potent drug in the treatment of diseases associated with free radical damage.

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules such as hydroxyl radical, hydrogen peroxide and superoxide anions, and are produced as by products in aerobic organisms and have been implicated in the pathology of a vast variety of human diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis. Antioxidants prevent the human system by neutralizing the free radicals interactively and synergistically. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity (Alam, Bristi and Rafiquzzaman, 2013).

The extract can be evaluated using different solvents to obtain further understanding of the antioxidant property because the antioxidant activity of plant origin is dependent on the type and polarity of the extracting solvent as well as on the test system and the

substrate to be protected by the antioxidant. Solvent extraction is frequently used for isolation of the antioxidants and both extraction yield and antioxidant activity of the extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity. For these reasons, comparative studies for selecting the optimal solvent providing maximum antioxidant activity are required for each substrate (Sarikurkcu, 2011).

However, the components responsible for antioxidant activity of the extracts are unclear. Future studies will be aimed at investigating the effects of different parts of *Aglaonema hookerianum* upon isolating and identifying the substances responsible for the antioxidant effects of the solvent extracts.

The extract also displayed significant cytotoxic activity as observed in the brine shrimp lethality test, which has been successfully used as a simple biological test to guide the fractionation process of plant extracts in order to detect antitumor compounds. The LC_{50} value was <1000 μg/ml so the extract can be regarded as a promising candidate for a plant-derived antitumor compound. This bioassay has good correlation with the human solid tumors cell lines. However, further and more specific bioassays are necessary in order to confirm these conclusions.

Chapter Six

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