Phytochemical and Pharmacological Investigations of Methanol Extract of *Bombax ceiba* Root

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

Submitted By:

Ishrat Jahan

ID: 2012-1-70-052

Department of Pharmacy

East West University

January 2016

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled **"Phytochemical and Pharmacological Investigations of Methanol Extract of *Bombax ceiba* Root"** is an authentic and genuine research work carried out by me under the guidance of **Nazia Hoque,** Senior lecturer, Department of Pharmacy, East West University, Dhaka.

…….

Ishrat Jahan
ID: 2012-1-70-052
Department of Pharmacy
East West University, Dhaka.

CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"Phytochemical and Pharmacological Investigations of Methanol Extract of *Bombax ceiba* Root"** is an authentic research work done by **Ishrat Jahan**, ID: 2012-1-70-052, in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

…….

Nazia Hoque
Senior Lecturer
Department of Pharmacy
East West University, Dhaka.

ENDORSEMENT BY THE CHAIRPERSON

"Phytochemical and Pharmacological Investigations of Methanol Extract of *Bombax ceiba* Root" is a n authentic research work done by **Ishrat Jahan**, ID: 2012-1-70-052 under the guidance of **Nazia Hoque**, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

……..

Dr. Shamsun Nahar Khan

Chairperson

Department of Pharmacy

East West University, Dhaka

ACKNOWLEDGEMENT

All praises are for the Almighty Allah for His mercy and blessings. I feel proud to express my heartiest regards and deep sense of gratitude to my reverend teacher and supervisor **Ms. Nazia Hoque**, Senior Lecturer, Department of Pharmacy, East West University, for her day to day mastermind direction, constant supervision and support, constructive suggestions, optimistic counseling, valuable criticism, keen interest and active encouragement to carry out this research work.

It gives me immense pleasure to express sincere appreciation and gratefulness to Professor **Dr. Shamsun Nahar Khan**, Chairperson, Department of Pharmacy, East West University for providing all possible help during the course of this research work.

It is also great pleasure for me to offer my deepest indebtedness to all of my respected teachers of the Department of Pharmacy, East West University for extending their helping hands whenever needed.

I also would like to extend my thanks to the Lab officers of the Department of Pharmacy, East West University for their help and assistance whenever needed.

I wish to thank my fellow researchers namely **Mir Mohammad Nakir Hossain**, **Faizul Hafiz** and **Sumiya Ferdous** for their endless cooperation and wholehearted inspiration throughout the period of the research work which enabled me to work in a congenial and comfortable atmosphere.

Finally, I express my sincere thankfulness to my family members for guiding me all through my life, including that for my research project.

Ishrat Jahan

ID: 2012-1-70-052

Dedicated to

"To my beloved Mother"

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Bombax ceiba a medicinal herb belonging to the Family- Bombacaceae also known as Red silk cotton tree (local name: Shimul) used as a folk medicine for the treatment of various diseases like nocturnal emission, diarrhea, muscular injury, acne, cold, wounds etc in this sub continent.

Different types of phytoconstituents such as sugar, steroid, flavonoids, alkaloids, terpenoids, tannins & saponins along with their structure & their applications have been reported from the leaf, bark, root, fruit, flower & seeds. These compounds have significant biological activities like anti-inflammatory, antimicrobial & antioxidant.

The study was designed for phytochemical investigations of methanolic extract of roots of *Bombax ceiba* & screening of their biological activities like, antimicrobial & antioxidant activities. The powdered roots of *Bombax ceiba* was extracted with methanol.

Phytochemical screening result showed the presence of carbohydrates, alkaloids, flavonoids, steroids. terpenoids, tannins & saponins. Some are present in a sufficient amount while others are in low amount.

The antimicrobial screening test was estimated by disc diffusion method. In antimicrobial activity investigation, methanolic crude extract of *Bombax ceiba* (roots) showed mild to moderate activity against the test bacteria. Two different concentrations: 300µg/disc & 600µg/disc of methanol extract of *Bombax ceiba* root was used in antibacterial test. Which is compared to kanamycin (30µg/disc) used as positive control in this study. The range of zone of inhibition was observed in between 7-13 mm depending on the concentration of the extract.

Another study was conducted to evalute the total antioxidant capacity of methanol extract of *Bombax ceiba* (roots) using aluminum chloride colorimetric method. The total flavonoid contents was 13.816±1.06 mg/g, as flavonoids have antioxidant property the present result suggests that the tested root extracts have antioxidant activity.

Key Words: *Bombax ceiba*, Phytochemical, Antimicrobial, Antioxidant.

CHAPTER

ONE

INTRODUCTION

1.1. Introduction

Plants and human are inseparable. Plants existed on the earth in the geological past form the early history of the earth. The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. From the early stages of human civilization, plants, especially medicinal plants have played a significant role for the welfare of human beings. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores of people of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75-80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects.. WHO defines medicinal plants in the following way "A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs" (Ghani, A. 1998 ; Khan, M. *et al*. 2001).

1.1.1. Medicinal Plant

A plant which has been used for medical purposes at one time or another, and which, although not necessarily a product or available for marketing, is the original material of herbal medicines is referred as medicinal plant. Plant materials treated according to traditional procedures to improve their safety and efficacy, to facilitate their clinical use, or to make medicinal preparations. Finished, labeled pharmaceutical products in dosage forms that contain one or more of the following: powdered plant materials, extracts, purified extracts, or partially purified active substances isolated from plant materials are herbal medicinal products.

Generations of skilled herbal practitioners, researchers and scholars have refined and tested the vast science of herbology, producing thousands of plant-based remedies that are safe and effective. The proper and judicious use of herbs is often successful in the

treatment of illness when other, more conventional medicines and methods fail. Herbs can be used to cleanse the bowels, open congested sinuses, help mend broken bones, stimulate the brain, increase libido, ease pain, aid digestion, and a thousand other purposes. Topically, herbs can repair damaged skin, soothe a wound, improve complexion, heal bruises and relieve aching muscles. Herbs demonstrate great versatility for the treatment of a broad variety of health needs (Medicinehunter 2014; WHO. 2015).

1.1.2. Importance of Medicinal Plant

Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples.

While medicinal plants are the actual plants themselves, plant medicines are preparations made from those plants. Plant medicines are the most widely used medicines in the world today. An estimated eighty percent (80%) of the world's population employs herbs as primary medicines. And while drugstore shelves in the US are stocked mostly with synthetic remedies, in other parts of the world the situation is quite different. In parts of Europe, for example, pharmacies dispense herbs prescribed by physicians.

For 5.1 billion people worldwide, natural plant-based remedies are used for both acute and chronic health problems, from treating common colds to controlling blood pressure and cholesterol. Not so long ago, this was true in the US as well. As late as the early 1950's, many of the larger pharmaceutical companies still offered a broad variety of plant-based drugs in tablet, liquid and ointment forms.

The efficacy of some herbal products is beyond doubt, the most recent examples being Silybum marianum (silymarin), Artemisia annua (artemesinin) and Taxus baccata (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some

established remedies, for instance, Ginkgo biloba for tinnitus, Hypericum perforatum is a reputed remedy for depression. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb.

Recently research has supported biological activities of some medicinal herbs. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease. Allamanda cathratica [allamandin], Elephatopus elatus [elephantpoin], Helenium autmnale [helenalin] Vernonia hymenlepis, Heliotropium indicum [Indicine-N-oxide], Daphne mezereum (mezerien) and Stereospermum suaveolans [laphacol] are medicinal plants that have shown significant tumor inhibiting effect (Medicinehunter. 2014; Worldpress. 2015).

1.1.3. Medicinal Plant in Ancient Time

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: Rhei rhisoma, camphor, Theae folium, Podophyllum, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra.

The 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. According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense.

The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (Centaurium umbellatum Gilib) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents.

Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. In the description of the plant toxic action, Theophrast underscored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants.

In his work "De re medica" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc.

In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic materia medica until the late Middle Ages and the Renaissance (Medknow Publications. 2012).

1.1.4. Medicinal Plant in 21st Century

For centuries people have used plants for healing. Plant products – as parts of foods or botanical potions and powders – have been used with varying success to cure and prevent

diseases throughout history. Written records about medicinal plants date back at least 5000 years to the Sumerians (Swerdlow, 2000) and archeological records suggest even earlier use of medicinal plants. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers (Verpoorte and Alfermann, 2000). The twentieth century became a triumph for the synthetic-chemistry-dominated pharmaceutical industry, which replaced natural extracts with synthetic molecules that often had no connection to natural products. The spectacular rise of the pharmaceutical industry had a tremendous impact on disease treatment and prevention, saved countless lives and became one of the outstanding achievements of the twentieth century. However, the benefits of modern drugs are felt primarily in developed countries, and developing countries continue to rely on ethnobotanical remedies as their primary medicines, leaving almost 75% of the world population without access to the modern healthcare products taken for granted in the West (Raskin, I. *et al*. 2002).

1.1.5. Present Drug from Medicinal Plant

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

sometimes, therapeutic efficiency, are applied as therapeutic means. In the major European producer and consumer of herbal preparations—Germany, rational phytotherapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs.

With the new Law on Drugs and Medical Devices dated September 2007 and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs (Medknow Publications. 2012).

Table 1.1: Some commonly used drugs derived from Plant Sources

Drug/Chemical	Action/Clinical Use	Plant Source
Acetyldigoxin	Cardiotonic	Digitalis lanata
Adoniside	Cardiotonic	Adonis vernalis
Allyl isothiocyanate	Rubefacient	Brassica nigra
Atropine	Anticholinergic	Atropa belladonna
Caffeine	CNS stimulant	Camellia sinensis
Camphor	Rubefacient	Cinnamomum camphora
Codeine	Analgesic, antitussive	Papaver somniferum

Demecolcine	Antitumor agent	Colchicum autumnale
Digitoxin	Cardiotonic	Digitalis purpurea
Ephedrine	Sympathomimetic, antihistamine	Ephedra sinica
Hyoscyamine	Anticholinergic	Hyoscyamus niger
Papavarine	Smooth muscle relaxant	Papaver somniferum
Quinidine	Antiarrhythmic	Cinchona ledgeriana
Reserpine	Antihypertensive, tranquillizer	Rauvolfia serpentina
Strychnine	CNS stimulant	Strychnos nux-vomica
Taxol	Antitumor agent	Taxus brevifolia
Theobromine	Diuretic, vasodilator	Theobroma cacao
Thymol	Antifungal (topical)	Thymus vulgaris
Vinblastine	Antitumor,Antileukemic agent	Catharanthus roseus
Vincristine	Antitumor,Antileukemic agent	Catharanthus roseus

(Taylor, L. 2000)

1.1.6. Future of Medicinal Chemistry

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of our National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified. Our group has also isolated several compounds, mainly from edible plant species or plants used as dietary supplements that may act as chemopreventive agents. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds (Balunas, M. and Kinghorn, A. 2005).

1.1.7. Economic Opportunities

Medicinal plants play an important role in the development of potent therapeutic agents. During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. However, the benefits of modern drugs are felt primarily in developed countries, and developing countries continue to rely on ethnobotanical remedies as their primary medicines, leaving almost 75% of the world population without access to the modern healthcare products taken for granted in the West. It is easy to overlook the fact that human medicines still contain Phytochemicals – valued at US$22 608 million in 1997 and projected to reach a value of US$30 688.5 million in 2002 – with prescription products and over-the-counter (OTC) herbal remedies each comprising approximately 50% of the market (McWilliams, 2014). The severed

bond between plants and health was felt not only in the area of medicines. By providing a 'pill option', the pharmaceutical industry also diminished the historical connection between food and the treatment of disease. 'An apple a day keeps the doctor away' is the advice one usually gets from a mother, not from a professional health organization. Plants are slowly making a comeback in several areas of human health (i.e. functional foods, dietary supplements and recombinant protein manufacturing) (Raskin *et al* 2002; Verma, S. and Sing S.P. 2008).

1.1.8. Medicinal Plant 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. In Bangladesh there are 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 a survey on "Traditional and industrial use and market Scenario of Medicinal plants in Bangladesh." conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh, 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. Another research aimed at documenting the 'Present Status and Market Scenario of Medicinal Plants' in Bangladesh 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 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 (Mpbd.info, 2014).

Table 1.2: List of Medicinal Plants in Bangladesh and their use

SL No	Local Name	Scientific Name	Family	Using part	Control Disease Name	Image of the Plant
1.	নিম	*Azadirachta indica*	Meliaceae	Root, leaf, bark	Skin disease, worm killer arthritis, insecticide, jaundice, antiviral.	

2.	বেল	*Aegle marmelos*	*Aegle marmelos*	Fruit	Dysentery diarrhea.	
3.	দূর্বা	*Cynodon dactylon*	Grominae	Leaf	Bleeding control, skin disease.	
4.	ঘৃতকুমারী	*Aloe indica*	Liliaceae	Extract of leaf	Headache, sexual disease, metabolic problem, fever.	
5.	পুদিনা	*Mentha viridis*	Lebiatae	Whole plant	Metabolic disorder, gastric.	

6.	বাসক	*Adhatoda vasica*	Acanthaceace	Leaf, root of plant	Cough, asthma, tuberculosis, cold, blood refine.	
7.	যষ্টিমধু	*Hydrangea arborescons*	Saxifrazaceac	Leaf flower fruit	Liver disease, adrenal peptic ulcer, hormonal disease, cold, throat pain.	
8.	সর্পগন্ধা	*Rauvolfia Serpentina*	Apocynaceae	Leaf and root.	Blood pressure, brain abnormal, dysentery diarrhea pain killer.	
9.	ব্রাম্মিশাক	*Becopa moniera*	Scrophulariaceae	Leaf	Heart disease, nurval pressure, asthma.	

10.	কুরচি	*Holarrhena antidysenterica*	Apocynaceae	Bark & Seed	Diarrhea, dysentery, worm killer constipation, intestinal weakness	
11.	কালমেঘ	*Andrographis pariculata*	Acanthaceae	whole plant	Metabolic problem, gastric, fever, worm killer, dysentery, liver disease, strengthens.	
12.	আকন্দ	*Calotropis procera*	Asclepiadaceae	Root, leaf. Bark flower extract of Leaf.	Ulcer, tooth pain chronic dysentery, cold, asthma	

13.	অনন্তমূল	*Hemidesmus indicus*	Asclepia-daceae	Root and whole plant	Strength increaser, apetiser, arthritis, diabetes.	
14.	আপাং	*Achyranthes Paniculata*	Amaranth aceae	whole plant	Dysentery, constipation, piles, arthritis, skin disease.	
15.	অর্জুন	*Terminalia arjuna*	Combreta ceae	Bark	Heart disease, diarrhea, piles, tuberculosis.	

(Yusuf *et al*. 2007)

1.1.9. Classification of Medicinal Plant

Many methods have been proposed to classify drugs and drug plants. Classifications can be based on the chemical nature or the therapeutic value of the plant product, the natural affinities of the various species or the morphology of the plant organ from which the drug is obtained. Hill (1952) proposed a morphological basis of classification. Generally it is found that the active principles are present in the storage organs of the plants, especially

in roots and seeds, and less in leaves, bark, wood or other plant parts. The amount of the chemical substances present in any specific organ is so small that it is difficult to give any biological significance to it. There may be some slight protective function, but most likely the action that is valuable to humans in the treatment of disease are merely waste products of plant metabolism (Hill, A.F. 1952).

1.1.10. Drug Discovery from Medicinal Plants

Despite the great synthetic diversity derived from the development of combinatorial chemistries and high-throughput screening methods over the past fifty years, natural products and related structures continue to be extremely important elements of pharmacopoeias. Looking forward, natural products and related structures are likely to become even more important for development of improved and new medicines, due to the variety of functionally relevant secondary metabolites of microbial and plant species whose chemical and genetic diversity are being revealed by ultra fast DNA sequencing and related genomics and bioinformatics tools.

Multivariate analyses and network modeling enable comprehensive identification and evaluation of natural product diversity and functionality; and when integrated with systems approaches, it is possible to profile molecular changes caused by mutation and by pathogens and other environmental stressors, and thus to predict the targets and mode(s) of action and toxicities of natural products and derivatives. Considerable synergy and benefit for the development of improved medicines and new drugs can come from linking these powerful scientific tools to robust ethnomedical and ethnobotanical studies of traditional medicines.

Inclusion of traditional medicines in development of 21st century treatment paradigms can help assure their convenience, acceptability and accessibility. Furthermore, pharmacological synergism, a principle employed by many traditional medicines lessens the likelihood of development of genetic resistance by the pathogen or disease against drug monotherapies. Synergy research inspired by a “reverse pharmacological approach”, could lead to a “new generation of phytopharmaceuticals”. The use of powerful ‘omics

technologies facilitates disentangling such complexity, metabolomics analyses enable profiling of major and minor metabolites and bioactive components that contribute to synergism; and computational approaches for analysis of multiple-activity networks have become powerful tools for defining the principal components of mixtures with synergistic modes of action, for prediction of drug metabolism and toxicity, and for high-throughput prioritizing of agent combinations. Data mining approaches to identify active compounds in mixtures of natural products are being developed and will be essential for the development of effective multiple-agent drugs from traditional medicines (Linh, T. N. *et al* 2013).

1.1.11. WHO's Policy on Herbal Medicines

The World Health Organization is fully aware of the importance of herbal medicines to many of its Member States and supports the use of medicinal plants and their products. In early 1978, the World Health Assembly, the WHO governing body, adopted a resolution on drug policies and management of medicinal plants, which recognized the importance of medicinal plants in the health care system. The World Health Assembly proposed coordinating efforts through the preparation of an inventory of medicinal plants, the development of criteria and methods for proving the safety and efficacy of medicinal plant products, and the dissemination of relevant information. In 1987, 1988 and 1989, three more resolutions were adopted covering the identification, evaluation, preparation, cultivation, utilization, regulation and conservation of medicinal plants.

Based on those resolutions, WHO's policy on herbal medicine may be summarized as follows:

(1) WHO is fully aware of the importance of herbal medicines for the health of a large number of the population in today's world. Herbal medicines are recognized as valuable and readily available resources, and their appropriate use is encouraged;

(2) To promote the proper use of medicinal plants, a comprehensive programme for their identification, evaluation, preparation, cultivation, recognition as valuable and readily available resources, and their appropriate use is encouraged;

(3) It is necessary to make a systematic inventory and assessment (pre-clinical and clinical) of medicinal plants; to introduce measures on the regulation of herbal medicines to ensure quality control of herbal products by using modern techniques, applying suitable standards and good manufacturing practices; and to include herbal medicines in the national standard or pharmacopoeia.

(4) As many of the plants that provide traditional and modern drugs are threatened with extinction, WHO endorses the call for international cooperation and coordination to establish programmes for the conservation of medicinal plants, to ensure that adequate quantities are available for future generations (WHO. 2015).

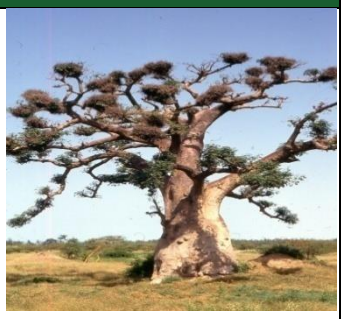
1.2. Plant Information

1.2.1. The Plant Family- Bombacaceae

Bombacaceae (Bombax, Baobab or Kapok family) is a small family of flowering plants which contains about 28 genera and 200 species. Plants of this family are perennial, deciduous and woody trees. They occur naturally throughout the tropical and subtropical regions of the world especially in tropical America. Many species grow to become large trees, reaching a height of 70 m. Additionally, some of these plants have considerable girth, so called "bottle trees" and their trunks are usually with buttresses at the base. Besides the great significance of Bombacaceae plants as ornamentals due to their large branches and brightly colored flowers, several genera are economically and commercially important, producing timber, edible fruits, vegetable oils or useful fibers, e.g., silk floss trees (Chorisia spp.) and Kapok (fibers of Ceiba fruits). The family is also noted for some of the softest hardwoods commercially traded, especially Balsa wood. The Baobabs (Adansonia spp.) are important icons in certain parts of Africa and Australia, noted for

their immensely stout trunk development which is a mechanism for enhancing water storage. Moreover, members of Bombacaceae found several folkloric medicinal uses in many countries due to their antipyretic, analgesic, anti-inflammatory, astringent, stimulant, diuretic, and antimicrobial properties (Refaat, J. *et al*. 2012).

Table 1.3: Different plants in Bombacaceae family

Genus	Common Name	Properties	Image
Adansonia digitata	Baobab tree, Dead rat tree	Antioxidant, Antipyretic, Antimicrobial, Antiviral, Analgesic	
Bombax glabra	Guiana chestnut, Malabar chestnut	Blood tonic, Help anemia, low blood pressure, fatigue and to generally build strength.	
Ceiba pentandra	Kapok	Anti-diabetic, Aphrodisiac,Antipyretic, Thermogenic, Diuretic, Emetic, Febrifuge, Purgative, Tonic, Demulcent & Astringent	

Ochroma pyrimidale	Balsa wood	Diuretic, Emetic, Emollient, Treats cold & cough	

(Mora, J.M. *et al*, 1999; Watson, L., and Dallwitz, M.J. 1992)

1.2.2. History

The great Swedish botanist, physician, and zoologist Carl Linnaeus (1707-1778), who laid the foundations of the modern biological scheme of binomial nomenclature and is considered the father of modern taxonomy, described and named this species in 1753 in his seminal work *Species Plantarum* (Hodel, D.R. and Weissich, P.R. 2012).

1.2.3. Botany

Red silk cotton tree (Bombax ceiba) is a tree with "a very striking feature in any landscape where it occurs, in the months of December, when it loses its foliage, and January, when it bursts into a blaze of scarlet flowers upon the naked branches" (Rameshwar, V *et al*. 2014).

1.2.4. Common Names

Simbal, Simul, Indian kapok, Katasavar, Shalmali, Red silk cotton tree, Silk-cotton tree.

Fig 1.1: *Bombax ceiba* tree

1.2.5. Taxonomical classification

Kingdom: Plantae

Division: Magnioliophyta

Class: Magniolipsida

Order: Malvales

Family: Malvaceae (Bombacaceae)

Genus: Bombax

Species: ceiba

Binomial name: *Bombax ceiba L.; Bombax malabaricum D.C.*

1.2.6. Morphology

Bombax ceiba is a lofty, deciduous tree upto 40 m tall and 6 m or more in girth with horizontally spreading branches and young stems covered with stout, hard prickles. The bark is pale ash to silver grey in color. Flowers are large in diameter, red in color and numerous with copious nectar. The fruits are brown capsule-like upto 15 mm long, filled with numerous black seeds which are irregular obovoid in shape, smooth and oily with dense silky hair. The fruit pulp is sweet and edible. Semal trees have compound leaves which are palmate in appearance. It is digitate, large, spreading, glabrous which has common petiole and the size of leaf is 15-30 cm long. The size of the leaflets varies from 10 cm to 20 cm. New leaves usually do not appear until flowering is over. The young stem and branches are covered with sharp, straight, stout prickles up to 1.2 cm long with woody conical bases.

Table 1.4: Organoleptic features of *Bombax ceiba* roots

Features	Observations
Shape	Cylindrical
Width	1-5 cm
Length	20-50 cm
Color	Peeled-pale yellow, unpeeled-yellowish brown to dark brown.
Odor	Faint and characteristic
Taste	Characteristic, free from bitterness.

(Chaudhary, P.H. *et al*. 2014)

Bark:

Bark *Bombax ceiba* of looks pale ashy to silver grey, 1.8 -2.5 cm thick, smooth up to middle age, becoming rough with irregular vertical cracks on older trees.

Fig 1.2: Bark of *Bombax ceiba*

Leaves:

Bombax ceiba tree has the compound leaves which is palmate in appearance. It is exactly appears as the palms appear in man. It is digitate, large, spreading, glabrous which has common petiole, and the size of leaf is 15-30 cm long. One leaf is composed of several leaflets. Five leaflets are common in one leaf but sometimes up to the seven leaflets could be found. The size of leaflets varies from 10 to 20 cm. generally the leaflets found in the centre are longer as in the fingers in palm. The leaflets are lanceolate, acuminate, more or less coriaceous and entirely glabrous.

Fig 1.3: Palmate Leaf

Flowers :

The bright red flowers, which appear in January to March, are large and conspicuous on the leafless trees. It presents a strikingly remarkable sight in winter and spring when the usually bare branches are covered with large, fleshy, red flowers. Birds are attracted to them, and are probably responsible for their pollination. These flowers form a scarlet carpet on the ground for few weeks (2-3 weeks) after dropping.

Fig 1.4: Flowers at branch tips.

The flower of *Bombax ceiba* are very showy, attractive and visible from long distances also. Because of its beautiful and attractive flowers, people like to plant it as the ornamental plant in the botanical garden, garden or as the avenue species. Flowers are numerous, large, 10-12.5 cm across. It clustered towards the ends of branches at the time of flowering. It has the thick, fleshy and cup shaped Sepals. It bears generally 5 petals in one flower which are 7.5-15 cm long oblong, recurved above, and fleshy, of bright crimson (rarely yellow or orange) color.

Capsule:

The pods are about 10-18 cm in length, oblong-oval in shape, locucidally 5 valved; valves woody, downy outside, lined with silky hairs within.

Fig 1.5: Woody capsule

Seeds:

Within the capsule it has many seeds which are obovoid, smooth, 6-9 mm long in size. These seeds are oily and surrounded by a thick mass of long silky hairs or floss, hence easily blown about by wind (Rajendra, K.C. 2007).

Fig 1.6: Seeds of *Bombax ceiba.*

1.2.7. Habitat and Distribution

It is widely found in temperate Asia, tropical Asia, Africa and Australia. In India, it can be found at altitudes upto 1500 m. In peninsular India, the tree is very common in the dry as well as moist deciduous forests and near rivers. The tree is a strong light-demander and fast growing. It grows best on deep sandy loams or other well-drained soils, particularly in valleys, in regions receiving 50 to 460 cm annual rainfall well distributed throughout the year.

Asia-Temperate

China: China -Fujian, Guangdong, Guangxi, Guizhou, Jiangxi, Sichuan, Yunnan

Eastern Asia: Taiwan

Asia-Tropical

Indian Subcontinent: Bhutan; India; Nepal; SriLanka

Indo-China: Cambodia; Laos; Myanmar; Thailand; Vietnam

Malesia: Indonesia; Malaysia; Papua New Guinea; Philippines

Australasia

Australia: Australia - Northern Territory, Queensland, Western Australia (Chaudhary, P.H. et. al. 2012; Rameshwar, V. *et al*. 2014).

1.2.8. Chemical Constituents

Root:

Sesquiterpene lactone isolated from the roots of a plant species identified as Salmaliamalbaricum (synBombax ceiba) was previously identified as hemigossylic acid lactone-7- methyl ether. 2D NMR experiments have shown that this was a new compound, isohemigossylic acid lactone-2-methyl ether. A detailed exploration of phytochemical properties along with the TLC ratios of various extracts of B. ceiba was also conducted which showed that the alcoholic and water extracts indicate the presence of alkaloids, flavonoids, glycosides, coumarins, proteins and amino acids.

Stem Bark:

It is reported to contain lupeol, β-sitosterol, shamimicin ceibanephthaquinone, simailn-A and simalin-B magniferin, epicatechin-7-O-b-xylopyranoside, epicatechin-3-O-bxylopyra-noside, shamiminol, stigmasta-3,5-diene, lupenone and opuntiol.

Flowers:

These contain β-sitosterol, β-sitosteril-β-D-glucoside, polysachharide-Dgalactose, hentriacontane, hantriacontanol, traces of essential oil, kaempferol, quercetin, L-arabinose, L-rhamnose.

Seeds:

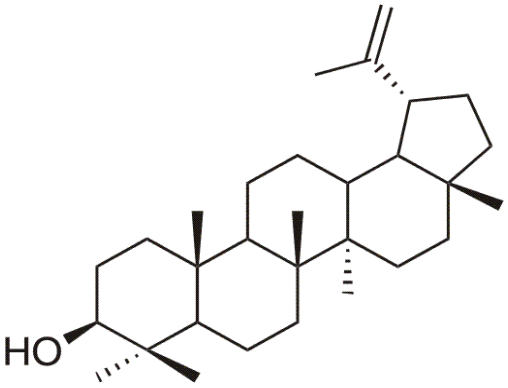
Seeds have n-hexacosanol, palmitic acid, octadecyl palmitate, gallic acid, tannic acid, 1-gallayl-β-glucose, and ethyl gallate, a mixture of α, β and ɤ- tocopherol.

Gum:

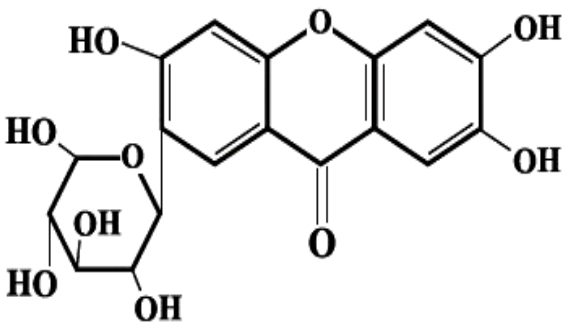
The gum obtained is reported to contain tannic acid, gallic acid, L-arabinose, D-galactose, D-glacturonic acid, aldobiuronic acid.

Leaves:

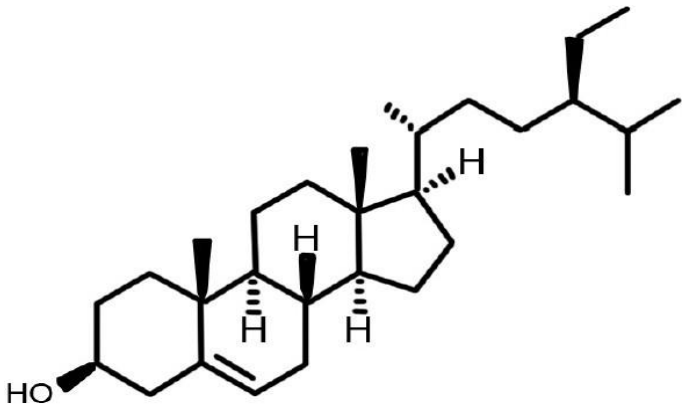
Leaves are reported to have crude protein, crude fiber, calcium, phosphorus, shamimin, mgniferin. (Rameshwar, V. *et al*. 2014; Shashi, C. *et al*. 2015)



A



B



C

Fig 1.7: Structures of (A) Lupeol, (B) Magneferin, (C) β-Sitosterol; Chemical constituents present in *B.ceiba*.

1.2.9. Medicinal Uses

- **Nocturnal Emission, Semen Problems:** Tender roots of *Bombax ceiba* took, cleaned and dried in shade. Then dried to make powders. This powder cures seminal disorder and nocturnal emission.
- **Anti-Inflammatory Activity:** the aqueous extract of *Bombax ceiba* showed a certain beneficial effects, suggesting this plant has a protective role on inflammatory bowel disease cases.
- **Anti-Diarrheal:** The bark juice was mixed with the bark juice of *Magnifera indica* and *P. guajava* and drunk to cure dysentery and intestinal spasm. The resin was also taken orally to treat worms and diarrhea; root juice was consumed to treat abdominal pain and gonorrhea.
- **Muscular Injury:** *B. ceiba* barks and roots were used to treat muscular injury.
- **Abortifacient:** 30g of seed powder of *B. ceiba* and about 10g Hing (*Ferula foeitida*) are used as an abortifacient.

- **Blood Purification:** Leaves of *Bombax ceiba* taken to grind with water and filtered. Drinking this works as blood purifier.
- **Leucorrhoea:** Taking *Bombax ceiba* root powder twice a day with water used as a treatment.
- **Over Bleeding in menstruation:** *Bombax ceiba* root powder (100gm), mulethi(50gm) , swarn geru (25gm) mixed, taking this powder twice a day with water or milk reduces this problem.
- **Acne, Skin Blemish and Pigmentation:** Thorny part of stem of semal tree taken to make paste with water. Applying on affected area also lightens scar marks due to boils, freckles, acne vulgarise and burns.
- **Cold and Cough:** Semal root powder mixed with black pepper and dry ginger powder. Taking in small amount to cure cold and cough.
- **Anthelmintics:** *Bombax ceiba* leaves exerted anthelmintic effects, a property that some plants and medicines have to help the body expel helminths or parasitic worms.
- **Wounds:** Paste of its bark can applied on wound.
- **Weakness:** From its flower green base part taken, cleaned and dried in shade. Then grinded to make powder. Mixing one spoon powder, honey (2 tbsp), desi ghee (1 tbsp) in milk and drink to recover weakness.
- **Improve Breast Milk:** The bark of semal root cleaned, dried and grinded to make powder. Taking twice to improve breast milk.
- **Leprosy:** The seeds and roots of *B. ceiba* were used in the treatment of leprosy.
- **Miscellaneous Uses:** Root bark extract was given as a tonic in case of sexual debility and also as nervine tonic. Root powder mixed with sugar candy and milk was taken to avoid impotency. The roots powdered with those of *Chlorophytum, Capparis sepiaria* and fruits of *Pedalium murex* were taken with water as a tonic for 7–8 days to calm body heat (Chaudhary, H.P. *et al.* 2012 ; Rameshwar, V. *et al*. 2014).

1.3. Screening Process

1.3.1. Phytochemical Screening

"Phyto" is the Greek word for plant. There are many "families" of Phytochemicals and they help the human body in a variety of ways. Phytochemicals may protect human from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many Phytochemicals can protect humans against diseases. Plant synthesizes a wide variety of chemical compounds which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites.

1.3.1.2. Phytochemistry

Phytochemistry is in the strict sense of the word the study of Phytochemicals. These are chemicals derived from plants. In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers.

1.3.2. Important Phytochemicals Present in Plant

1.3.2.1. Alkaloids

Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Also some synthetic compounds of similar structure are attributed to alkaloids. Beside carbon, hydrogen and nitrogen, molecules of alkaloids may contain sulfur and rarely chlorine, bromine or phosphorus.

- **Distribution of Alkaloids in Plants**

Alkaloids are generated by various living organisms (bacteria, fungi, plants, and animals), especially by higher plants – about 10 to 25% of those contain alkaloids. The

alkaloids content in plants is usually within a few percent and is inhomogeneous over the plant tissues. Depending on the type of plants, the maximum concentration is observed in the leaves (black henbane), fruits or seeds (Strychnine tree), root (*Rauwolfia serpentina*) or bark (cinchona). Furthermore, different tissues of the same plants may contain different alkaloids.

- **Biological Role of Alkaloids**

The role of alkaloids for living organisms which produce them is still unclear.

1. Initially it was assumed that the alkaloids are the final products of nitrogen metabolism in plants, and urea in mammals. Later it was shown that alkaloid concentrations vary over time and this hypothesis was refuted.

2. Most of the known functions of alkaloids are related to protection. For example, aporphine alkaloid liriodenine produced by the tulip tree protects it from parasitic mushrooms. In addition, presence of alkaloids in the plant prevents insects and chordate animals from eating it.

3. Some animals adapted to alkaloids and even use them in their own metabolism.

4. Besides, such alkaloid-related substances as serotonin, dopamine and histamine are important neurotransmitters in animals.

5. Alkaloids are known to regulate plant growth.

- **Applications of Alkaloids**

1. In Medicine:

Medical use of alkaloid plants has a long history, and thus when the first alkaloids were synthesized in the 19th century, they immediately found application in clinical practice. Many alkaloids are still used in medicine, usually in the form of salts, including the following:

Table 1.5: Alkaloids that are used in medicine	
Alkaloid	**Action**
Atropine, scopolamine, hyoscyamine	Anticholinergic
Colchicine	Remedy for gout
Physostigmine	Inhibitor of acetyl cholinesterase
Quinidine	Antiarrhythmic
Emetine	Antiprotozoal agent
Vinblastine, vincristine	Anticancer

Many synthetic and semi synthetic drugs are structural modifications of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side effects. For example, naloxone, an opioid receptor antagonist, is a derivative of thebaine which is present in opium.

2. In Agriculture:

Prior to the development of a wide range of relatively low-toxic synthetic pesticides, some alkaloids, such as salts of nicotine and anabasine, were used as insecticides. Their use was limited by their high toxicity to humans.

3. Use as Psychoactive Drugs:

Preparations of plants containing alkaloids and their extracts, and later pure alkaloids have long been used as psychoactive substances. Cocaine and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as psilocybin, dimethyltryptamine and ibogaine) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers. There are alkaloids that do not have strong psychoactive effect themselves, but are precursors for semi-synthetic psychoactive drugs. For example,

ephedrine and pseudoephedrine are used to produce methcathinone (ephedrine) and methamphetamine.

1.3.2.2. Flavonoids

Flavonoids (or bioflavonoid), also collectively known as Vitamin P and citrin, are a class of plant secondary metabolites.

- **Classification**

According to the IUPAC nomenclature, they can be classified into:

1. Flavonoids, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure (examples: quercetin, rutin)

2. Isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure

3. Neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

- **Distribution of Flavonoids in Plants**

Flavonoids are widely distributed in plants fulfilling many functions.

- **Biological Roles of Flavonoids**

1. Flavonoids are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals.

2. Flavonoids secreted by the root of their host plant help Rhizobia in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule

3. They protect plants from attacks by microbes, fungi and insects.

- **Potential for Biological Activity of Flavonoids**

1. *In vitro* studies of flavonoids have displayed anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities. Flavonoids (both flavonols and flavanols) are most commonly known for their antioxidant activity *in vitro*.

2. Consumers and food manufacturers have become interested in flavonoids for their possible medicinal properties, especially their putative role in prevention of cancers and cardiovascular diseases. Although physiological evidence is not yet established, the beneficial effects of fruits, vegetables, tea, and red wine have sometimes been attributed to flavonoid compounds rather than to known micronutrients, such as vitamins and dietary minerals.

3. Cancer: Physiological processing of unwanted flavonoid compounds induces so-called Phase II enzymes that also help to eliminate mutagens and carcinogens, and therefore may be of value in cancer prevention. Flavonoids could also induce mechanisms that may kill cancer cells and inhibit tumor invasion. UCLA cancer researchers have found that study participants who ate foods containing certain flavonoids, such as catechins found in strawberries and green and black teas; kaempferol from brussel sprouts and apples; and quercetin from beans, onions and apples, may have reduced risk of obtaining lung cancer.

4. Diarrhea: A study done at Children's Hospital & Research Center Oakland, in collaboration with scientists at Heinrich Heine University in Germany, has shown that epicatechin, quercetin and luteolin can inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl^- transport inhibiting cAMP stimulated Cl^- secretion in the intestine.

5. Capillary Stabilizing Agents: Bioflavonoids like rutin, monoxerutin, diosmin, troxerutin and hidrosmin have potential vasoprotective proprieties still under experimental evaluation.

1.3.2.3. Saponins

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species.

Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. A ready and therapeutically relevant example is the cardio-active agent digoxin, from common foxglove.

- **Distribution of Saponins in Plants**

Saponins have historically been understood to be plant-derived, but they have also been isolated from marine organisms. Saponins are indeed found in many plants, and derive their name from the soapwort plant (Genus Saponaria, Family Caryophyllaceae), the root of which was used historically as a soap. Saponins are also found in the botanical family Sapindaceae, with its defining genus Sapindus (soapberry or soapnut), and in the closely related families Aceraceae (maples) and Hippocastanaceae (horse chestnuts; ref. needed). It is also found heavily in gynostemma pentaphyllum (Genus Gynostemma, Family Cucurbitaceae) in a form called gypenosides, and ginseng (Genus Panax, Family Araliaceae) in a form called ginsenosides. Within these families, this class of chemical compounds is found in various parts of the plant: leaves, stems, roots, bulbs, blossom and fruit. Commercial formulations of plant-derived saponins- e.g., from the soap bark (or soapbark) tree, Quillaja saponaria, and from other sources- are available via controlled manufacturing processes, which make them of use as chemical and biomedical reagents.

- **Medical Uses of Saponins**

There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. There is evidence of the presence of saponins in traditional medicine preparations, where oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid (and obviation of any toxicity associated with the intact

molecule). But as is often the case with wide-ranging commercial therapeutic claims for natural products: the claims for organismal/human benefit are often based on very preliminary biochemical or cell biological studies; and mention is generally omitted of the possibilities of individual chemical sensitivity, or to the general toxicity of specific agents, and high toxicity of selected cases. While such statements require constant review (and despite the myriad web claims to the contrary), it appears that there are very limited US, EU, etc. agency-approved roles for saponins in human therapy. In their use as adjuvants in the production of vaccines, toxicityassociated with sterol complexation remains a major issue for attention. Even in the case of digoxin, therapeutic benefit from the cardiotoxin is a result of careful administration of an appropriate dose. Very great care needs to be exercised in evaluating or acting on specific claims of therapeutic benefit from ingesting saponin-type and other natural products.

1.3.2.4. Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. Tannins have molecular weights ranging from 500 to over 3,000. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine.

- **Distribution of Tannins in Plants**

The compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also in growth regulation.

- **Medicinal Uses of Tannins**

1. Tannins may be employed medicinally in antidiarrheal, hemostatic, and antihemorrhoidal compounds. The anti-inflammatory effect of tannins helps control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhea is

also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine.

2. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally.

3. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more.

4. Tannins are beneficial when applied to the mucosal lining of the mouth. Tannins can also be effective in protecting the kidneys.

5. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds.

6. Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue.

7. Tannins are used indirectly as molluscicides to interrupt the transmission cycle of schistosomiasis.

8. Tannins have shown potential antiviral, antibacterial and antiparasitic effects.It is believed that tannins isolated from the stem bark of Myracrodruon urundeuva are of neuroprotective functions capable of reversing 6-hydroxydopamine induced toxicity. The plant has shown promising futures for therapeutic use, which may be of benefit to neuro disease patients. Souza *et al*. discovered that the tannins isolated from the stem bark also has the anti -inflammatory and antiulcer potency on rodents, showing a strong anioxidant property for possible therapeutic applications.

9. Foods rich in tannins can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron resulting in a pathological increase in total body iron stores.

1.3.2.5. Terpenoids

Terpenoids are the most numerous and structurally diverse plant natural products. Basic unit of most secondary plant metabolites, including terpenes, consists of isoprene, a simple hydrocarbon molecule. The term terpene usually refers to a hydrocarbon molecule while terpenoid refers to a terpene that has been modified, such as by the addition of oxygen.

- **Distributions of Terpenoids in Plants**

They found the highest concentrations of terpenoids in heartwood, lowest in outer sapwood and moderate levels in the inner sapwood.

- **Medicinal Uses of Terpenoids**

1. Terpenoids have also shown antimicrobial activities. This is important due to the increase in antibiotic resistant bacteria, which is occurring globally and at an alarming rate.
2. Addition of terpenoids into livestock feed may replace conventional antibiotic addition, which in turn would slow the rate of antibiotic resistance in bacteria.
3. Cumene (isopropylbenzene) is a terpene that has been used in bioremediation studies.
4. Taxol (diterpene), a well known anticancer agent provides systematic advantages *in vitro* cell culture methods such as the ability to manipulate plant environment, control of cell growth, and regulation and extraction of metabolic products.
5. Pharmaceutical and food industries have exploited them for their potentials and effectiveness as medicines and flavor enhancers (Zwenger, S. and Basu, C. 2008).

1.3.2.6. Plant Steroids

Plant steroids are types of natural organic compounds found in plants. The most biologically prominent plant steroid is brassinolide ($C_{28}H_{48}O_6$), which is important to the development of plant cells and promoting the plant's growth. It is part of a larger class of plant steroids called brassinosteroids. Brassinolide is synthesized from

campesterol ($C_{28}H_{48}O$), another plant steroid that is part of a group of similar steroid compounds called phytosterols. Other examples of phytosterols, also commonly called plant sterols, include beta-sitosterol ($C_{29}H_{50}O$) and brassicasterol ($C_{28}H_{46}O$) (Markley, J. 2015).

- **Distributions of Steroids in Plants**

They are present in small quantities in many fruits, vegetables, vegetable oils, nuts, seeds, cereals and legumes.

- **Medicinal Uses of Plant Steroids**

1. Plant sterols/stanols included with a heart healthy eating plan may reduce your risk for heart disease. The sterols/stanols work by blocking the absorption of cholesterol in the small intestine. This lowers the low density cholesterol known as the 'bad' cholesterol (LDL) by 6-15%, without lowering the good cholesterol known as the high density cholesterol (HDL).
2. A special group of compounds called cardioactive steroids [better known as cardiac glycosides] deserves special mention. *Digitalis purpurea*, *Stropanthus gratus* and *Urginea indica* are reputed remedies as cardiac tonics. Clinically they find application in congestive cardiac failure. All of them contain steroid saponins as active constituents.
3. *Smilax officinalis* is medicinal herb that is known to contain steroid like compounds-saponin glycosides and according to some researchers actually the herb contains male hormones. It also has been used in herbal medicine as an anti-inflammatory agent in curing arthritis and rheumatism.
4. Coriander sativum (commonly known as fennel) also contains phyto- estrogens and has shown promise in treating premenstrual syndrome.
5. Withaferin-A is the most important group of Withanolide and has shown promise as potent anti cancer agent. The Withanolides are basically steriod lactones and various types have been isolated.

6. Oral contraceptives & sex hormones are derived from *Disogenin.* It is extracted from medicinal herbs as well as prepared commercially (Cleveland Clinic. 2009 ; Singh A.P, Sandhu A.S).

1.3.3. Antioxidant Activity Test

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the monophenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates,

and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

Free radicals are highly charged and active particles which are made of unstable molecules or atoms due to their single and unbalanced electrons. The common free radicals are oxygenreactive species (ROS), namely, super oxide radical, hydroxyl radical, and peroxyl radical which can be internally produced by cellular metabolism, inflammation by immune cells and externally by radiation, pharmaceuticals, hydrogen peroxide, toxic chemicals, smoke, alcohol, oxidized polyunsaturated fats and cooked food. They are unstable and through chain reaction can attack vital biomolecules (DNA, lipids, proteins) in cells and body fluids. They also weaken the cells in our bodies leaving us vulnerable to disorders and diseases such as arteriosclerosis, coronary heart disease, stroke, hypertension, emphysema, diabetes, cataracts, rheumatoid arthritis, nephritis, Alzheimer disease, cancer, AIDS, etc. Aging process is also a result of the oxidation by free radicals in the body. They are formed naturally, both internally by metabolism and externally by chemicals. These include alcohol consumption, drugs, toxic metals, emotional stress, smoking, pesticides, herbicides and air pollutants.

Fortunately, nature provides us with plenty of "protecting molecules" or the so called "antioxidants" which can trap or destroy free radicals and subsequently protect us from damage due to the oxidative stress. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (byproducts) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, macular degeneration, diabetes, cancer etc are all contributed by oxidative damage.

1.3.3.1. Natural Antioxidants

There are two groups of natural antioxidants.

- The first group is our body enzymes such as superoxide dismutase (SOD), catalysts, glutathione peroxidase. Wheat and barley grain products are rich in SOD.

- The other group is nutrient antioxidants which are vitamin E, vitamin C and beta-carotene (the pre-form of vitamin A).

In addition, there are still numerous other antioxidants such as bioflavonoids, carotenoids (such as lutein and lycopene) and phenolic compounds. Selenium is also an important mineral antioxidant. Selenium is commonly found in onions, garlic, mushrooms, whole grain cereals, particularly in the wheat germ and rice bran.

1.3.3.2. The Antioxidant Process

Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. That is why there is a constant need to replenish our antioxidant resources. How they work can be classified in one of two ways:

1. Chain Breaking-

When a free radical releases or steals an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs -- either the radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E, or it simply decays into a harmless product.

2. Preventive-

Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxides prevent oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion.

They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron. The effectiveness of any given antioxidant in the body depends on which free radical is involved, how and where it is generated, and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other systems it could have no effect at all. Or, in certain circumstances, an antioxidant may even act as a "pro-oxidant" that generates toxic oxygen species.

1.3.3.3. Free Radicals, Antioxidants & Health

Prevention is better than cure. The more toxic chemicals which produce free radicals we obtain, the more antioxidants in our diet we have to consume for the detoxification. We should be healthy and free from disease by preventing our body from any oxidative damage. In order to protect our better health or slow our aging, we should carefully avoid those factors which increase the production of free radicals, and daily eat more fresh vegetables and fruits, at least one pound or 400 grams, as well as sufficient amounts of nuts, seeds and pulses. Plant food contains a wide range of phytochemicals and antioxidants, which are protecting agents against free radicals. Beta-carotene is common in dark green, yellow, orange and red vegetables and fruits such as carrots, pumpkin, tomatoes, spinach, peppers, watercress, broccoli, cherries, peaches, papaya, watermelon and apricots. Several tropical fruits and vegetables are good sources of antioxidants.

- Vitamin C: Found in all fresh fruits and vegetables, particularly citrus fruits, melon, strawberries, leafy green vegetables and tomatoes.

- Vitamin E: Found in all whole-grain cereals including brown rice, oats, whole meal bread, wheat germ, soya beans, cold-pressed vegetable oils, nuts and seeds, parsley, broccoli and asparagus. In conclusion, if we can control free radicals, then we can reduce the degenerative diseases. Vegetarians have an advantage over non-vegetarians, because they eat a large variety of fresh vegetables, fruit and whole grains.

1.3.4. Antimicrobial Screening

The main objective of performing the antibacterial screening is to determine the susceptibility of the pathogenic microorganisms to test compound which, in turn is used to selection of the compound as a therapeutic agent. In general, antimicrobial screening in-vitro is undertaken in following two steps:

- **Primary Assay**

It is essentially a qualitative or semi qualitative test that indicates the sensitivity or resistance of microorganisms to the compound. However this technique cannot be used to distinguish between bacteriostatic and bactericidal agents. The primary assay can be performed *in vitro* by disk diffusion assay method, which includes:

a) Plate Diffusion Test

b) Streak Test

The plate diffusion test utilizes different concentrations of a test compound absorbed on sterile filter paper disks on the same plate whereas the streak test permits the determination of the antibacterial effect of a test compound on several microorganisms simultaneously and is suitable for the estimation of the spectrum of the activity. However, the plate diffusion test is commonly used.

- **Secondary Assay**

It quantifies the relative potency such as minimum inhibitory concentration (MIC). The lowest concentration of an antimicrobial agent required to inhibit the growth of the microorganisms *in vitro* is referred to as minimum inhibitory concentration (MIC). It is done by serial dilution technique (Reiner, R. 1982).

1.3.4.1. Antimicrobial Drug

Antimicrobial drug/Antibiotics are the greatest contribution at the present century at therapeutic. Antibiotics are special kind of chemotherapeutic agent usually obtained from

living organism. The term chemotherapeutic agent mean "All chemical substance that destroy all kind of cell wall such as bacterial cell wall, viral cell wall even human cell wall. "Antibiotics one kind of chemotherapeutic agent, but it does not destroy the human cell wall, it destroy the bacterial & viral cell wall. So all antibiotics are chemotherapeutic agent but all chemotherapeutic agents are not antibiotic. The word antibiotic come to refer to a metabolic of one microorganism that is very small amount is detrimental or inhibitory to their microorganism. The term antibiosis was first defined by vuillemin in 1889.The first systematic search for & study of antibiotics made by "Gratia & both about 1924. In 1929 Alexander Fleming discovers one kind of Antibiotics named by penicillin from the penicillium tree. Characteristics of Antibiotic: To be useful as chemotherapeutic agent antibiotics must have the following qualities:

(1) They should have the ability to destroy or inhibit many different species of pathogenic microorganism.

(2) They should prevent the ready development of resistant forms of the parasites.

(3) They should not be produced undesirable side effects in the host, such as sensibility or allergic reaction, never damage or irritation of the kidneys & gastrointestinal tract (G.I.T).

(4) They should not eliminate the normal microbial flora of the host. (Cui, J. *et al.* 2011)

1.3.4.2. Classification of Antibiotics

Antibiotic drug are classified in several way: For example, some are bactericidal & other are bacteriostatic.Here the term Bactericidal mean "Stop the Bacterial growth & it also kill the bacteria", and bacteriorstat mean "stop the bacterial growth but can not kill the bacteria."

Antibiotic may be grouped on the basis of chemical structure.

I) Sulfonamide & relative drugs

2) Diaminopyrimidines.

3) Quinolones

4) ß- lactam antibiotics

5) Nitromemzene derivatives

6) Amino glycosides.

7) Polypeptide Antibiotics.

8) Nitrofuran derivatives etc.

1.3.4.3. Antibiotics & Their Mode of Action

The major points of attack of antibiotics on microorganism include:

1) Inhibition of cell wall synthesis. Example: Penicillin, Bacitracin.

2) Damage to the cytoplasmic membran. Example: Ploymxins, Hamycin.

3) Inhibition of nucleic acid & protein synthesis. Example: Tetracyclines, Clidamycin.

4) Inhibition of specific enzyme systems. Example: Pyridine, Pyrimidine.

5) Interfere with DNA synthesis. Example: Acyclovir.

6) Interfere with intermediary metabolism. Example: Sulfonamides, PAS (Para amino salicylic acid).

1.3.4.4. Misuses of Antibiotics

1) Treatment of Untreatable Infection: The majority of the diseases causes by viruses will not respond to anti-infective agent. Thus antimicrobial therapy of measles, chickenpox, mumps & upper respiratory infection-90% is totally ineffective.

2) Therapy of fever of undetermined origin.

3) Improper doses.

4) Reliance on chemotherapy with omission of surgical drainage.

5) Lack of adequate bacteriological information.

Factors To Be Consider In Selecting Antibiotics Drug:

1) Clinical symptoms & site of infection.

2) Identity of the pathogen-samples for laboratory analysis

3) Drug toxicity.

4) Cost

5) Prior history drug allergy.

1.3.4.5. Rational Use of Antibiotics

1) Use appropriate antibiotics with adequate bacteriological information.

2) Antibiotics should be used in proper dose & for appropriate duration.

3) Combination of antibiotic should be used where single drug is an ineffective or to overcome the chance of microbial resistance to antibiotics.

4) A bactericidal antibiotics should not be used with a bacteriostatic antibiotic at the same time (antibiotic antagonism).

5) Broad spectrum antibiotics should not be used indiscriminately.

6) Consider the patient condition during selection of antibiotics (e.g. in renal failure ciprofloxacin is contraindicated).

7) Should not use the newer member of group of antibiotics so long as the currently used drug is effective.

1.4.1. Objectives

In order to achieve these aims, the following research objectives have been identified:

1. Phytochemical screening
2. Antioxidant activity study
3. Antimicrobial activity study

1.4.2. Study Area

The research was carried out in the Pharmacognosy Lab, Microbiology Lab, Chemistry Lab and Pharmacology Lab of Department of Pharmacy, East West University, Dhaka.

1.4.3. Data Collection

All the relevant data has been collected from two types of sources:

1. Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.

2. Secondary sources: various publications like journals, papers, documents and websites.

1.4.4. Research Protocol

1. Selection, identification, collection, drying and grinding of roots.

2. Extraction of the powders with methanol and collection of extract.

3. Phytochemical analysis of the root extract.

4. Antioxidant activity determination.

5. Anti Microbial activity determination.

6. Studying and comparing the results obtained.

1.4.5. Information Processing and Analysis

The data and the results collected were reviewed, compared, processed and organized. Some tests were repeated to be sure of the results. Some data were analyzed into flow charts and statistical tables where possible.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review

The whole plant or different parts of *Bombax ceiba* plants have been showed various pharmacological activities.

2.1. Whole Plant/ Root

2.1.1. Antioxidant Activity

The antioxidant activity of a methanol extract of *B.ceiba* was evaluated using several antioxidant assays, in terms of its: (i) ability to scavenge DPPH (1, 1-diphenyl-2-picryl-hydrazyl) and hydroxyl free radicals; (ii) action against lipid peroxidation (in rat liver microsomes and soy bean phosphatidylcholine liposomes), induced by ascorbyl radicals and peroxynitrite; and (iii) effect on myeloperoxidase activity. The cytotoxicity was monitored through the mitochondrial activity in the Vero cell line.

Recently, methanol extract of root showed high amounts of phenolics (30.9% w/w) and tannins (15.45%w/w) and a very good DPPH scavenging activity (EC_{50} 15.07 µg/ml) in a dose-dependent manner as well as dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) with a maximum absorbance of 1.11 at a concentration of 500 µg of extract. Furthermore, acute study in human healthy volunteers showed a significant ($p < 0.05$) rise in total antioxidant status at the end of 4 h after administration of 3 g root powder (Jain, V. and Verma S.K. 2014).

2.1.2. Antimicrobial and Antibacterial Activity

Plant extracts (methanol and aqueous) were assayed for their activity against multi-drug resistant *Salmonella typhii*. Strong antibacterial activity was shown by the methanol extracts of *Salmalia malabarica.* Plant or plant parts were collected, dried, homogenized and extracted in two organic solvents viz. methanol and acetone. The antibacterial activity against *Klebsiella pneumoniae* was done by agar disc diffusion method. The activity was compared with standard antimicrobials Amikacin and Piperacillin.

Ethanolic extract of *Bombax ceiba* was evaluated as strong anti-helicobacter pylori activity. The minimum inhibitory concentration of ethanolic extract ranged from 0.64 to 10.24mg/ml.

Recently, the methanolic extract of root in five concentrations (50, 25, 12.5, 6.25 and 3.12 mg/ml) has shown a significant dose-dependent antibacterial potential in *in vitro* agar well diffusion assay. Zone of inhibition (mm diameter) obtained at the highest concentration of methanolic extract (50 mg/ml) for Grampositive bacteria *Staphylococcus aureus*, *B. subtilis* and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* was 17.0, 16.0, 17.16 and 17.10, respectively (Shashi, C. *et al* 2015 ; Jain, V. and Verma, S.K. 2014).

2.1.3. *In-vitro* Anti-Inflammatory Activity

In-vitro anti-inflammatory activity of extracts of *B.ceiba* was assessed by Human Red Blood Corpuscles (HRBC) membrane stabilizing method with slight modifications. The blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension in normal saline was made. Diclofenac potassium (50mcg/ml) was used as standard. The reaction mixture (4-5 ml) consisted 2 ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (1000 mcg/ml) in normal saline and 0.5 ml of 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56ºC for 30 min. and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm using visible Spectrophotometer. The experiment was performed in triplicates. The control represents 100% lyses.

The present in-vitro study is a preliminary evaluation of anti-inflammatory activity of *B. ceiba* and demonstrated that folk medicine of *B. ceiba* can be used to cure the inflammation. Further research work to analyze in-vivo anti-inflammatory activity of *B.*

ceiba on animal models and to isolate the phytoconstituents responsible for anti-inflammatory activity are ongoing. Isolation of the respective phytoconstituents from *B. ceiba* directs to investigate the possible mechanism of action at cellular level which may become a useful approach to develop natural bioactive products. The present study suggests that *B. ceiba* would serve as a source for the discovery of novel anti-inflammatory agents (Anandarajagopal, K. *et al*. 2013).

2.1.4. Inhibitory Effects on Fatty Acid Syntheses

Fatty acid syntheses (FAS) had been found to be over express and hyperactive in most cancers. Pharmacological inhibitors of FAS activity preferentially repress cancer cell proliferation and induce cancer cell apoptosis without affecting nonmalignant fibroblasts. These made FAS an excellent drug target for cancer therapy. The FAS activity is the lowest in gastric cancer cell N87 (15.91 ± 3.61 U/mg protein) and the highest in lung cancer cell A549 (127.36 ±10.14 U/mg protein). The cancer cell A549 was used as a cell model to test the inhibitory effort of flavonoid extracts on FAS. The minimum inhibitory concentration of *B. ceiba* Linn was 247.98µg/ml (Rameshwar, V. *et al*. 2014).

2.1.5. Cytotoxicity

Aqueous extracts of the plants were screened for their cytotoxicity using the brine shrimp lethality test. The present study supports that brine shrimp bioassay is simple reliable and convenient method for assessment of bioactivity of medicinal plants and lends support for their use in traditional medicine (Rameshwar, V. *et al*. 2014).

2.2. Bark

2.2.1. Anti-Diabetic Activity

Bombax ceiba bark extract was evaluated for its hypoglycemic and hypolipide-mic potential through normal and streptozotocin-induced diabetic rats administered with graded oral doses (200, 400, 600 most effective to cause significant ($p<0.001$) hypoglycemic and/or hypolipidemic effects on streptozotocin mg/kg/day) for 21 days.

The results showed that a dose of 600 mg/kg of *B. ceiba* extract is the -induced diabetic rats. This dose also significant-ly ($p<0.001$) lowered the total cholesterol and triglyceride level in severely diabetic rats. Phytochemical and GC-MS studies confirmed the presence of the triterpenoid compounds in the extract, which may account for its significant hypoglycemic activity. The present study thus provides a scientific rationale for the traditional use of this plant in the management diabetes (Bhavsar, C. and Talele, G.S. 2013).

2.2.2. Antiangiogenic Activity

A methanol extract of the stem barks of *B. ceiba* was found to exhibit a significant antiangiogenic activity on *in vitro*tube formation of human umbilical venous endothelial cells (HUVEC).Bioactivity-guided fractionation and isolation carried out on this extract identified lupeol as an active principle. At 50 and 30µg/ml, lupeol showed a marked inhibitory activity on HUVEC tube formation while it did not affect the growth of tumor cell lines such as SK-MEL-2, A549 and B16-F10 melanoma (Rameshwar, V. *et al*. 2014).

2.2.3. Anti-Obesity

The extract of stem bark of *Bombax ceiba* Linn.has significant anti-obesity potential against HFD induced experimental obesity, possibly due to modulation of FAS and PTP-1B signaling in Wistar rats due to the presence of active flavanoids and lupeol respectively (Rameshwar, V. *et al*. 2014).

2.2.4. Hypotensive Activity

Shamimin along with lupeol [lup-20 (29) en-3b-ol],which possesses potent hypotensive activity, have been isolated from *B. ceiba* stem bark. BCBMM [filtrate from BCBM (Methanolic extract of defatted stem bark)] one of the most active fractions has revealed its adverse effects on heart, liver and kidneys of mice at the dose of 1000 mg/kg/d (Rameshwar, V. *et al*. 2014).

2.3. Leaves

2.3.1. Analgesic Activity

Mangiferin, 2-beta-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one, obtained directly from methanolic extracts of *B. ceiba* leaves demonstrated strong antioxidant activity (EC(50) 5.8 (+/-) 0.96 mμg/ml) using DPPH assay. The acetyl and cinnamoyl derivatives were found to be less active than mangiferin whereas methyl and 3, 6, 7-trimethylether tetraacetate derivatives were inactive implying that for antioxidant activity, free hydroxyl groups and catechol moiety are essential. Moreover, mangiferin showed hepatoprotective activity against carbon tetrachloride induced liver injury further supporting the free radical scavenging property in the in vivo system. Additionally, crude plant extracts and purified mangiferin failed to exhibit acute anti-inflammatory activity whereas, extracts displayed significant analgesic effect in acetic acid-induced writhing and hot plate tests in mice. Using naloxone, it was revealed that plant extract induced analgesia was independent of the opioid receptor; whereas, mangiferin demonstrated significant interaction with the receptor at a peripheral site, with a slight contribution at the neuronal level (Rameshwar, V. *et al*. 2014).

2.3.2. Hypotensive and Hypoglycaemic Activity

Shamimin, a C-flavonolglucoside from *B.ceiba* leaves showed significant potency as a hypotensive agent at the doses of 15 mg/kg, 3 mg/kg, 1 mg/kg and significant hypoglycaemic activity at 500 mg/kg in Sprague Dawley rats (Rameshwar, V. *et al*. 2014).

2.3.3. Antipyretic

The methanol extract of *Bombax ceiba* leaves was investigated for the antipyretic activity in rats. MEBM possessed significant antipyretic activity in Baker's yeast-induced pyrexia. Phytochemical tests showed the presence of steroids, carbohydrates, tannins, triterpenoids, deoxy-sugars, flavonoids and coumarin glycosides (Rameshwar, V. *et al*. 2014).

2.4. Flowers

2.4.1. Hepatoprotective Activity

The hepatoprotective activity of a methanolic extract of flowers of *B. ceiba* (MEBC) was investigated against hepatotoxicity produced by administering a combination of two anti-tubercular drugs isoniazid (INH) and rifampicin (RIF) for 10 and 21 days by intraperitoneal route in rats. MEBC were administered at three graded dose i.e. 150, 300 and 450 mg/kg i.p. 45 min prior to anti-tubercular challenge for 10 and 21 days. MEBC was evident in all doses as there was a significant decrease in alkaline phosphatase (ALP), alanine transaminases (ALT), aspartate transaminases (AST) and total bilirubin levels, but increase in the level of total protein in comparison to control. MEBC significantly decreased the level of TBARS (thiobarbituric acid reactive substances) and elevated the level of GSH (reduced glutathione) at all doses as compared to control. The results obtained from the analysis of biochemical parameters and histopathological studies, resulted in the conclusion that the MEBC were not able to completely revert the hepatic injury induced by INH and RIF, but it could limit the effect of INH and RIF to the extent of necrosis (Rameshwar, V. *et al*. 2014).

2.5. Fruits

2.5.1. Diuretic Effects

The present study was aimed to investigate the diuretic effects of aqueous and crude ethanol extracts of *Bombax ceiba* L. fruits (family, Bombacaceae) using acute model in rats. A single individual dose of aqueous and ethanol extract of *B. ceiba* fruit (200 mg/kg and 400 mg/kg, p.o., each), frusemide and hydrochlorothiazide, (25 mg/kg, p.o., each) as reference diuretic drugs, were administered orally to dehydrated rats. Control group rats were fed with normal saline (25 ml/kg, p.o.). All rats were caged in metabolic cages in pairs and their urine output was monitored at 5 and 24 h intervals. Both extracts significantly increased the urine output in higher doses. Although, the onset of this diuretic action was gradual (within 5 h), it lasted throughout the studied period (up to 24 h). Further, the intensity of diuresis induced by aqueous extract (400 mg/kg) in 5 h was almost similar to that of frusemide and hydrochlothiazide. Aqueous extract of *B. ceiba*

fruit also caused marked increase in urinary Na^+ and K^+ levels. However, the routine urinalysis showed non-significant alterations in pH and specific gravity by either dose of crude extracts of *B. ceiba* fruits. These effects demonstrate possible diuretic actions of *B. ceiba* fruit extracts and support its folklore use in various urinary ailments. Further studies need to be done to characterize the active phytoconstituents from fruits. (Jalalpure, S. S. and Gadge, N. B. 2011)

Table 2.1: Summary of Literature Review

Activity	Plant Part	References
Antioxidant activity	Plant, Root	Jain, V. and Verma S.K. 2014
Anti-inflammatory activity	Plant	Anandarajagopal, K. *et al*. 2013
Anti-diabetic activity	Bark	Bhavsar, C. and Talele, G.S. 2013
Analgesic activity	Leaves	Rameshwar, V. *et al*. 2014
Antiangiogenic activity	Bark	Rameshwar, V. *et al*. 2014
Anti-obesity	Bark	Rameshwar, V. *et al*. 2014
Antipyretic	Leaves	Rameshwar, V. *et al*. 2014
Antimicrobial and antibacterial activity	Plant, Root	Shashi C *et al* 2015 ; Jain, V. and Verma S.K. 2014
Cytotoxicity	Plant	Rameshwar, V. *et al*. 2014
Diuretic effects	Fruits	Jalalpure, S. S. and Gadge, N. B. 2011

Fatty acid syntheses inhibition	Plant	Rameshwar, V. *et al*. 2014
Hypotensive and hypoglycaemic activity	Leaves	Rameshwar, V. *et al*. 2014
Hepatoprotective activity	Flowers	Rameshwar, V. *et al*. 2014
Hypotensive activity	Bark	Rameshwar, V. *et al*. 2014

CHAPTER THREE

MATERIALS & METHODS

3. Materials and Methods

3.1. Preparation of the Plant Sample

3.1.1 Collection and Proper Identification of the Plant Sample

At first with the help of a comprehensive literature review *Bombax ceiba* was selected for this investigation. The roots were collected from Aftabnagar, Dhaka, Bangladesh.

3.1.2 Plant Material Preparation

The roots of the plants were collected in fresh condition. It was sun-dried and then, dried in an oven at reduced temperature (not more than 50^0C) to make suitable for grinding purpose. The coarse powders were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

3.1.3 Extraction Procedure

The powdered plant materials were submerged into methanol in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent.

3.1.4 Filtration of the Extract

After the extraction process the plant extract was filtered with sterilized cotton filter. The cotton was rinsed with methanol and fitted in a funnel. The filtrate was collected in a beaker. Then again it was filtered and this time what man's filter paper was used for getting more clear extract which would be useful making the sample more concentrated in rotary evaporator technique. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper and was prepared for rotary evaporator.

Fig 3.1: Rotary Evaporator

3.1.4.1 Procedure

After the filtration process two parts were obtained namely 'residual part' and filtered part or filtrate". The filtered part, which contains the substance soluble in methanol, was putted into a 1000ml round bottom flask and then the flask was place in a rotary evaporator. The evaporation was done at 50 temperatures. The number of rotation per minute was selected as 120 rpm. The pressure of the vacuum pumper machine was 6bar. The water flow through the distillation chamber was also provided in a satisfactory flow rate. When the evaporation seemed to be satisfactory, then the methanol extract was collected in a 50mL beaker. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. The evaporator flask was rinsed by methanol. Then the beaker was covered with aluminum foil paper and kept for 60 minutes. Finally the concentrated methanol plant extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigations.

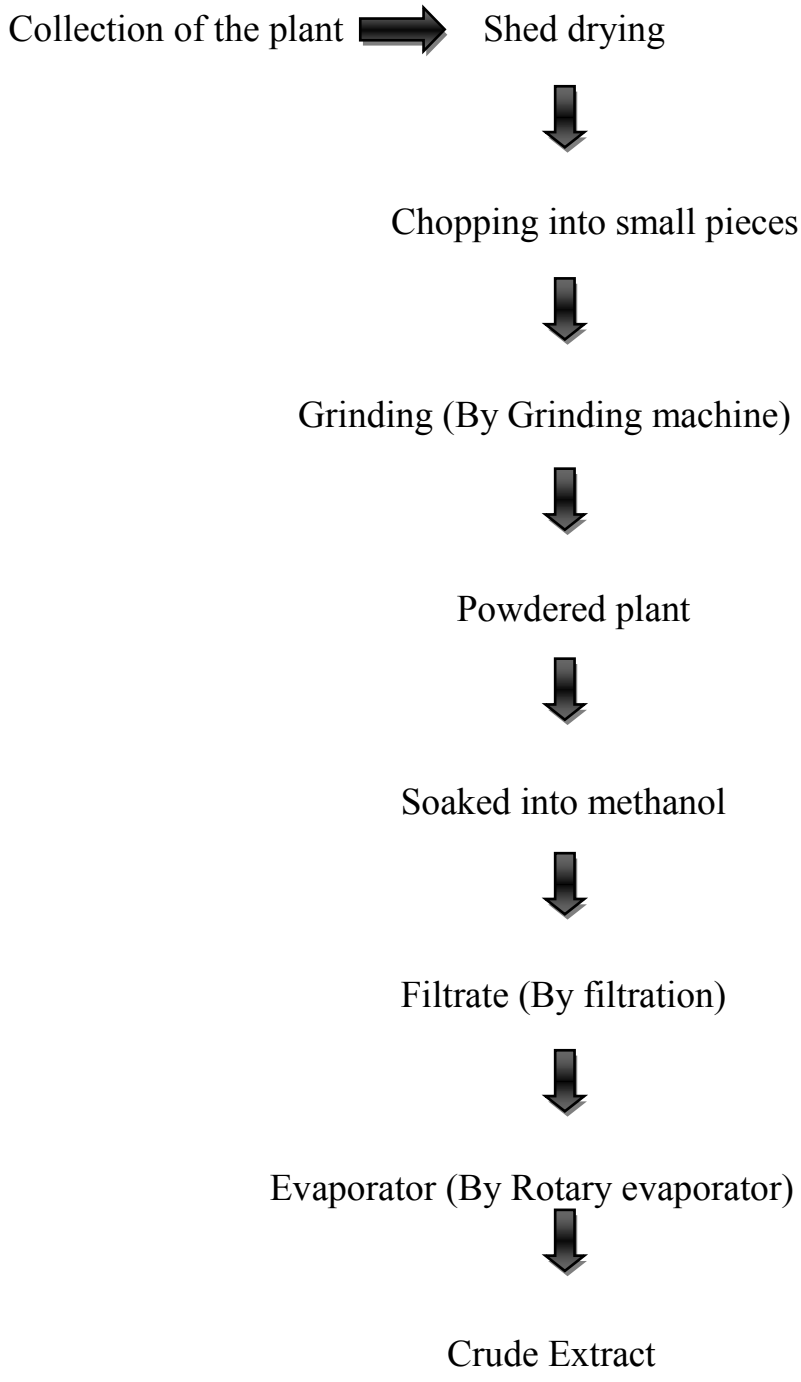


Fig 3.2: Schematic Presentation of the Crude Preparation From The Plant

3.2. Theory of Phytochemical Screening

3.2.1. Principle

Modern medicines are intimately related to chemistry and detailed examinations of active principles of plants and other products from an essential part of it. The healing properties of the plants are due to the presence of physiologically active chemical compounds inside the plant materials. Knowledge of chemical constituents of plants would further be valuable to those interested in the expending area of chemotaxonomy, to those interested in biosynthesis and to those interested in deciphering the actual value of folkloric remedies. This phytochemical investigation or screening is an evaluatory process for the detection of plant constituents through chemical analysis; phytochemical screening is co-related with phytochemical study. The compounds isolated through phytochemical study are applied on treated animal to find out the pharmacological effect either beneficial or toxic and thus toxic plants are separated.

In this research work methanolic extracts of *Bombax ceiba* was screened for Carbohydrates, Saponins, Flavanoids, Tannins, Steroids, Alkaloids and Tarpinoid that have pronounced medical value.

Carbohydrates are important food reservoir for the plant and food stuffs for men and other animals. Of special pharmaceutical importance is the fact that sugar units with a wide variety of other compounds to form glycosides. Tannins are widely distributed in plant kingdom and they have pronounced astringent and antimicrobial properties. Most of the plant derived drugs of our present world are alkaloid containing. Alkaloids have remarkable physiologic and pharmacologic properties. Plant materials containing saponins have long been used in many parts of the world for their detergent properties. As saponins are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids. They also have hemolytic properties and some of them constitute important medicines.

3.2.2 Materials Used

3.2.2.1 Equipments

- Test tube
- Watch glass
- Holder
- Burn

3.2.2.2 Reagents and Chemicals

Table 3.1: Reagents and Chemicals required for Phytochemical Screening	
Tests	**Reagents**
Carbohydrate Test	a) Benedict Test b) Barfoed's Test
Alkaloid Test	a) Wagner's reagent (solution of iodine in KI) b) Hager's reagent (saturated solution of picric acid)
Flavonoid Test	Conc. HCl
Steroid Test	Chloroform, Conc. H_2SO_4
Tannin Test	$FeCl_3$ (5%)
Terpenoid Test	Conc. H_2SO_4
Saponins Test	Olive oil

3.2.2.3. Test Compounds

Methanol extract of *Bombax ceiba*.

3.2.3. Preparation of Sample Solution

Small amount of dried, decolorized extracts were appropriately treated to prepare sample solution and then subjected to various phytochemical tests.

3.2.4. Phytochemical Tests

Various phytochemical tests which were performed under heading of phytochemical screening are mentioned below:

i. **Tests for Carbohydrats**:
   a) Benedict's Test: To 0.5ml of filtrate, 1ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 5 min. a characteristic colored ppt. indicates the presence of sugar.
   b) Barfoed's Test: To 1ml of extract, 3ml of Barfoed's reagent is added & heated on a water bath for 2 min. Red ppt. indicates presence of sugar.

ii. **Tests for Alkaloid:** A small volume of each extract was neutralized by adding 1 or 2 drops of dilute H_2SO_4 . This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation was observed:
   a) Hager's reagent: Formation of yellow crystalline precipitate indicated the presence of alkaloids.
   b) Wagner's reagent: Formation of brownish-black ppt indicated the presence of alkaloids.

iii. **Test for Flavanoids:** A few drops of conc. HCl was added to a small amount of an extract. Immediate development of a red color indicated the presence of flavanoid.

iv. **Test for Steroids:** A small amount of extract was added with 2 ml of chloroform, then 1 ml of conc. H_2SO_4 was carefully added from the side of the test tube. In presence of steroids ,a red color was produced in chloroform layer.

v. **Test for Tannins:** About 0.5 ml of extract was stirred with 10 ml of distilled water. Production of a blue, blue-black, green or blue-green coloration or precipitation on the addition of $FeCl_3$ (5%) reagent was taken as evidence for the presence of tannins.

vi. **Test for Terpenoids:** About 2ml of dichloromethane was added to the sample, then conc. H_2SO_4 was carefully added, reddish brown coloration is the evidence for the presence of terpenoids.

vii. **Test for Saponin:** About 1ml of extract was added to 10ml of distilled water, production of foam with the addition of olive oil indicates the evidence of saponins. (Ghani, A. 2003; Chakraborty, D.D. *et al*. 2010)

Carbohydrate **Alkaloid**

Benedict's Test & Barfoed's Test Hager's Reagent & Wagner's Reagent

Flavonoid **Steroid** **Tannin** **Terpenoid** **Saponin**

Fig 3.3: Phytochemical Tests Performed in the Laboratory

3.3. *In vitro* Determination of the Antioxidant Activities

3.3.1. Principle

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. The most common reactive oxygen species (ROS) include superoxide (O_2.) anion, hydrogenperoxide (H_20_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO.) and peroxynitrite anion (ONOO.). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome. In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti carcinogenic etc. They were also suggested to be a potential iron chelator. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties.

3.3.2. Methods

The antioxidant activity of the methanol extracts of *Bombax ceiba* were determined by using the flavonoid contents.

3.4. Determination of Flavonoid Contents

3.4.1. Principle

Aluminum chloride colorimetric method was used for flavonoids determination. 1.5 ml extract mixed with methanol, distilled water and sodium nitrate (5%w/v). Then the mixture was incubated and aluminum chloride (10%) was added into it. After further incubation, sodium hydroxide (4%) was taken and distilled water added. The absorbance of the reaction mixture was measured at 695 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/ml of sample.

3.4.2. Reagents

- Aluminum Chloride ($AlCl_3$)
- Sodium Nitrate ($NaNO_2$)
- Sodium hydroxide (NaOH)
- Methanol
- Quercetin (Analytical or Reagent grade)

3.4.3. Preparation of 10% Aluminum Chloride ($AlCl_3$) Solution

10 gm of $AlCl_3$ was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.4.4. Preparation of Standard Solution

Quercetin stock solution of concentration 5µg/µl was prepared by dissolving 0.025 gm of quercetin into 5 ml of methanol. The experimental concentrations from the stock solution were prepared by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled methanol	Final volume
100	100 µl	-	4.90 ml	5 ml
50	-	2 ml (100µg/ml)	2 ml	4 ml
25	-	2 ml (50µg/ml)	2 ml	4 ml
12.5	-	2 ml (25µg/ml)	2 ml	4 ml

3.4.5. Preparation of Extract Solution

1.50 gm of plant extract were taken and dissolved into methanol. The concentration of the solution was 1mg/ml of plant extracts.

3.4.6. Experimental Procedure

- 1.5 ml extract or standard of different concentration solutions were taken in different test tubes and methanol were added into the test tubes.
- After that, 6ml distilled water was added with 0.45 ml Sodium Nitrate (5%w/v) .
- The mixture was incubated for 6 minutes, then 0.45 ml aluminum chloride (10%) taken and again incubated for 6 minutes.
- Then, 6 ml Sodium hydroxide (4%) was taken and 0.6ml distilled water added.
- The absorbance of the reaction mixture was measured at 695 nm with UV/Visible spectrophotometer

3.5. *In vitro* Antibacterial Screening

3.5.1. Principle

Bacteria are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* bacterial growth. This ability may be estimated by any of the following three methods.

i) Disc diffusion method
ii) Serial dilution method
iii) Bioautographic method

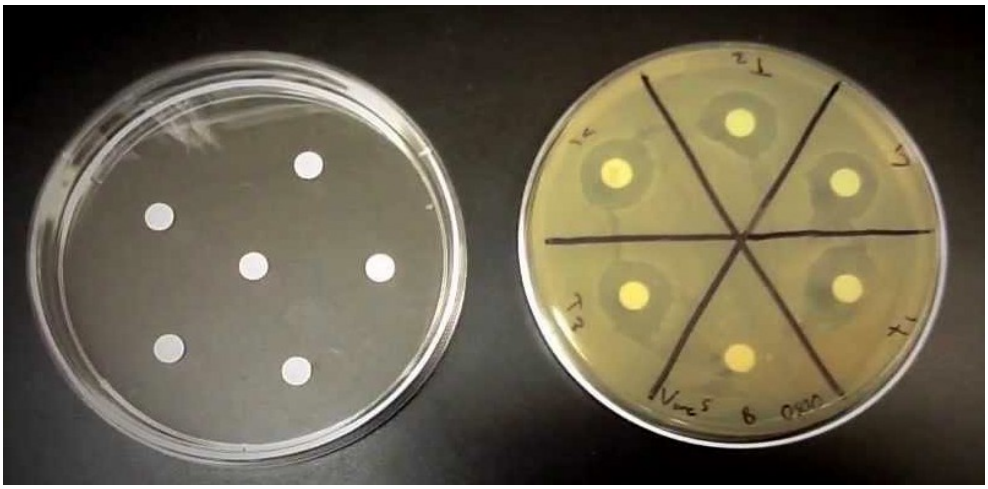


Fig 3.4: Disc diffusion method.

But there is no standardized method for expressing the results of antimicrobial screening. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculums volume, culture medium composition, P^H and incubation temperature can influence the results.

Among the above mentioned techniques the disc diffusion is a widely accepted *in vitro* investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method.

3.5.2. Principle of Disc Diffusion Method

Solutions of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4 ^{0}C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change of test materials concentration in the media surrounding the discs.

The plates are then incubated at 37 ^{0}C for 24 hours to allow maximum growth of the organisms. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms and a clear, distinct zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter.

The experiment is carried out more than once and the mean of the readings is required.

In the present study the crude methanol extract of *Bombax ceiba* was tested for antibacterial activity by disc diffusion method.

3.5.3. Experimental work

Apparatus and Reagents

- Filter paper discs
- Petridishes
- Inoculating loop
- Sterile cotton
- Sterile forceps
- Spirit burner
- Micropipette
- Screw cap test tubes
- Nosemask and Hand gloves
- Laminar air flow hood
- Autoclave
- Incubator
- Refrigerator
- Nutrient Agar Medium
- Ethanol
- Chloroform

3.5.3.1. Test Materials

- **Test samples**

  Crude methanol extract of *Bombax ceiba*

3.5.3.2. Test Organisms

The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both Gram positive and Gram-negative organisms were taken for the test and they are listed below:

3.5.3.3. List of Test Bacteria

1. *Vibrio parahemolyticus*
2. *Vibrio mimicus*
3. *Staphylococcus aureas*
4. *Escherichia coli*

3.5.4. Culture medium and their composition

The following media is used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms.

Nutrient Agar Medium

Ingredients	Amount
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s. to	100 ml
P^H	7.2 ± 0.1 at 25^0C

Nutrient Broth Medium

Ingredients	Amount
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm

Distilled water q.s.to	100 ml
P^H	7.2 ± 0.1 at 25^0C

Fig. 3.5: Nutrient Agar in Culture Bottle

Muller-Hunton medium

Ingredients	Amounts
Beef infusion	30 gm
Casamino acid	1.75 gm
Starch	0.15 gm
Bacto agar	1.70 gm
Distilled water q.s. to	100 ml
P^H	7.3 ± 0.2 at 25^0 C

Tryptic soya broth medium (TSB)

Ingredients	Amounts
Bacto tryptone	1.7 gm
Bacto soytone	0.3 gm
Bacto dextrose	0.25 gm
Sodium chloride	0.5 gm
Dipotassium hydrogen Phosphate	0.25 gm
Distilled water q.s. to	100 ml
P^H	7.3 ± 0.2 at 250c

Nutrient agar medium (DIFCO) is used most frequently for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

3.5.6 Preparation of Culture Medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The P^H (at 25 ^{0}C) was adjusted at 7.2 – 7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure/ sq. inch at 121 ^{0}C for 20 minutes. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

3.5.7. Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glasswares were sterilized by autoclaving at a temperature of 121 ^{0}C and a pressure of 15-lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

3.5.8.1. Preparation of Subculture

In an aseptic condition under laminar air hood cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37 ^{0}C for their optimum growth. These fresh cultures were used for the sensitivity test.

3.5.8.2. Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petri dishes. The petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

3.5.9. Preparation of Discs

3.5.9.1. Standard Discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Amoxicillin (10µg/disc) standard disc was used as the reference.

3.5.9.2. Blank Discs

These were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.

3.5.9.3. Preparation of Sample Discs with Test Samples

50 mg of each test samples were dissolved in 2 ml of ethanol to obtain the concentration 25 mg/ml in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Fig. 3.6: Filter paper discs.

3.5.9.4. Preparation of Sample Discs

Methanol extracts of *Bombax ceiba* were tested for antimicrobial activity against a number of both gram positive and gram negative bacteria. The amount of sample per disc was 100 µg and 1 mg.

3.5.9.5. Preparation and Application of the Test Samples

Sample discs were prepared by adding 20 µl of the test solutions to the sterile filter paper discs. The discs were then allowed to dry for sufficient period of time until complete

evaporation of the solvent. The test samples were applied to previously sterilized discs using adjustable micropipette under aseptic conditions.

3.5.10. Diffusion and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours.

3.5.11. Determination of Antimicrobial Activity by the Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Bauer, A.W. *et al*. 1966).

CHAPTER FOUR

RESULT

&

DISCUSSION

4. Results & Discussion

4.1. Result of Phytochemical Analysis

Table 4.1: Qualitative Analysis of the Phytochemicals in Methanol Extract of *Bombax Ceiba* (Roots)

Name	Availability
1. Alkaloids	++
2. Carbohydrates	+
3. Flavonoids	++
4. Saponins	++
5. Terpenoids	+++
6. Tannins	+++
7. Steroids	+++

Symbol (+++) indicates presence in high concentration, Symbol (++) indicates presence in moderate concentration, symbol (+) indicates low concentration.

4.2. Result of Antioxidant Activity

4.2.1. Total Flavonoid contents

Total flavonoid contents of *Bombax ceiba* were determined by using the aluminum chloride colorimetric method and were expressed as quercetin equivalents per gram of root extract. The total flavonoid contents of the test fractions were calculated using the standard curve of quercetin ($y= 0.0098x - 0.0364$; $R^2 = 0.9724$). Then the absorbance at 695 nm was determined. These data were used to estimate the flavonoid contents using a standard curve obtained from various concentration of quercetin. Total flavonoid contents were expressed as mg of quercetin equivalent.

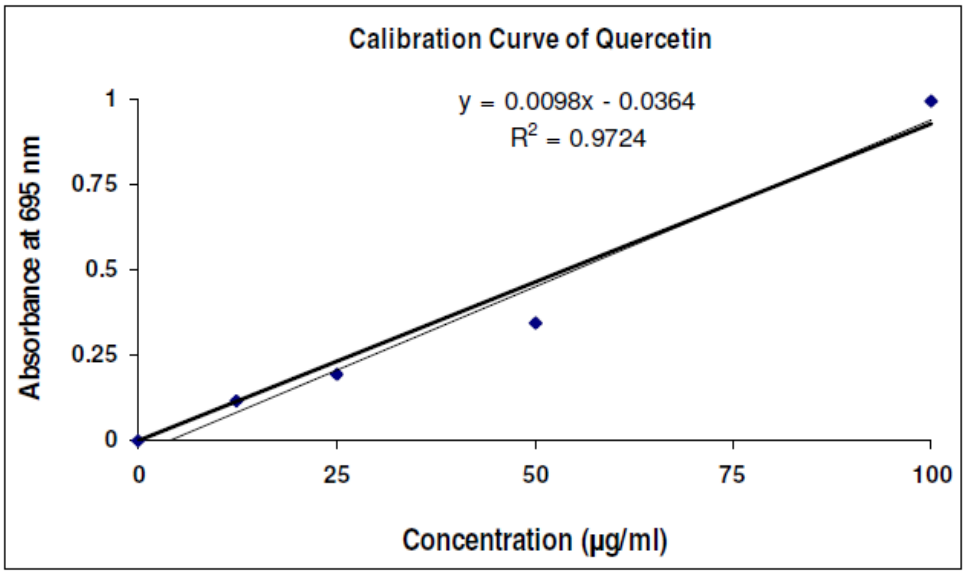


Fig.4.1: Calibration Curve of Quercetin

Table 4.2 : Determination of Total Flavonoid Assay

Sample	Concentration	Absorbance	Total Flavonoid Contents	Average (Mean ±Standard Deviation)
Methanol Extract of *Bombax ceiba*	1mg/ml	0.102	14.122	13.816±1.06
	1mg/ml	0.096	13.510	

The total flavonoid contents 13.816±1.06 mg/g equivalent of quercetin rules are present from duplicate data and mean ± standard deviation.

4.3. Result of Antimicrobial Screening

The methanol extract (300 µg/disc & 600 µg/disc) of *Bombax ceiba* showed antibacterial activity against several gram negative and gram positive bacteria.

Table 4.3 : The Antibacterial Activity (*In vitro*) of *Bombax Ceiba* Methanol Extract & Kanamycin Discs

Serial No.	Name of the Test Organism	Diameter of the Zone Of Inhibition (in mm)		
		Methanol Extract		Standard (Kanamycin)
		300 µg/disc	600 µg/disc	30 µg /disc
Gram Positive Bacteria				
1.	*Staphylococcus aureaus*	_	7mm	34mm
Gram Negative Bacteria				
1.	*Vibrio parahaemolyticus*	13mm	8mm	20mm
2.	*Eschericia coli*	7mm	7mm	30mm
3.	*Vibrio mimicus*	8mm	7mm	13.5mm

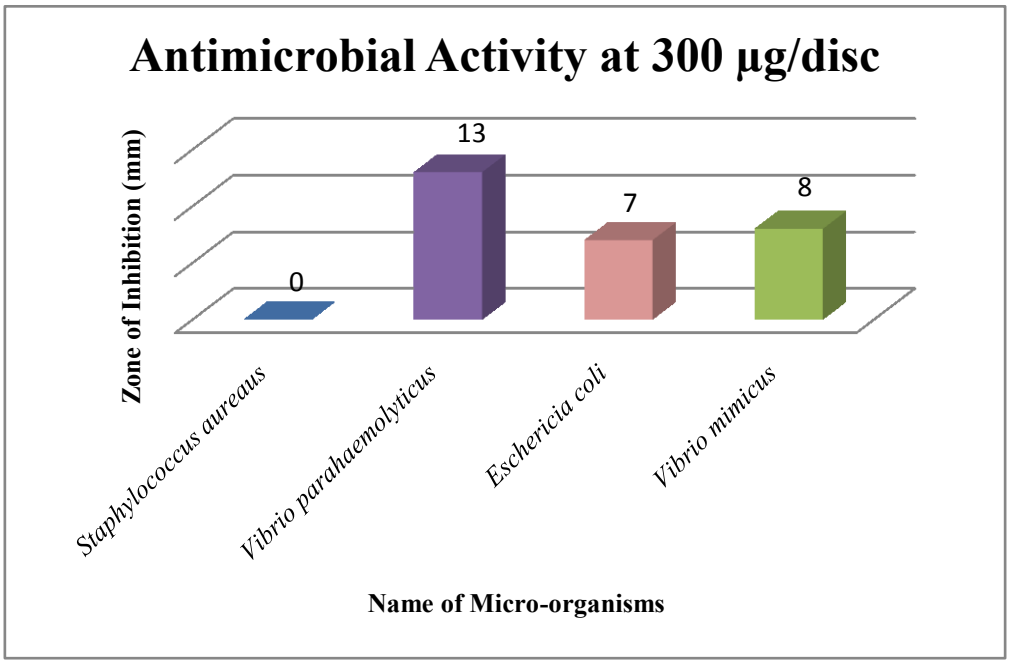


Fig 4.2: Zone of Inhibition of Different Micro-organisms at 300µg/disc

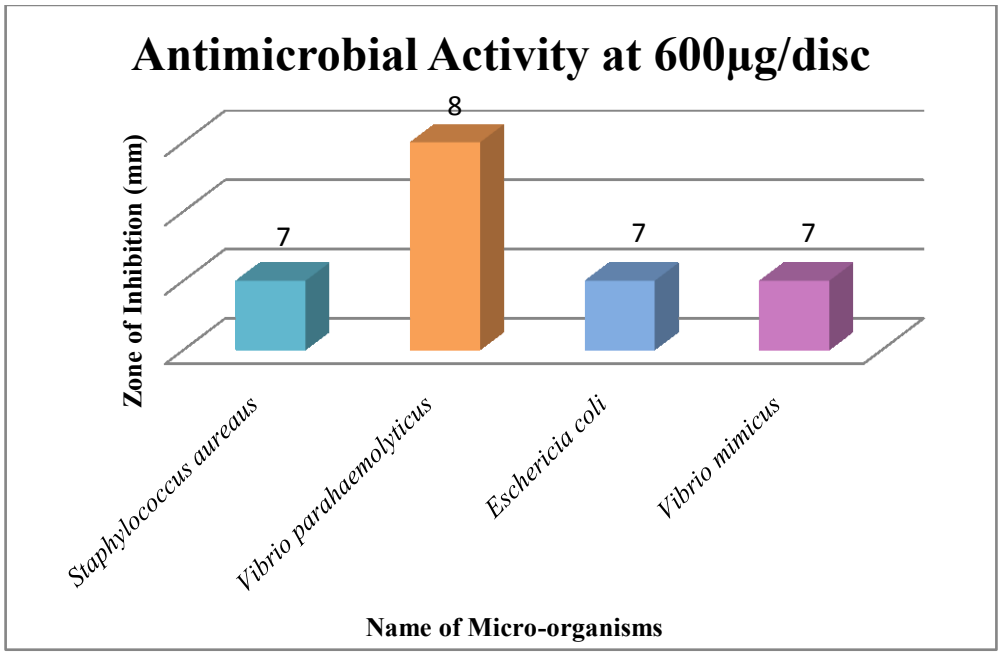


Fig 4.3: Zone of Inhibition of Different Micro-organisms at 600µg/disc

4.4. Discussion

Phytochemical screening showed that the methanol extract of *Bombax ceiba* root was rich in phytochemical constituents such as terpenoids, tanins and steroids. Thus further research is needed to work out the active medicinal compounds present in this extract; used for the treatment of various types of diseases.

Recently, antioxidant activities of *B. ceiba* root using DPPH radical scavenging and reducing power have assessed. Methanol extract of root showed high amounts of phenolics, tannins and a very good DPPH scavenging activity in a dose-dependent manner as well as dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation). Furthermore, acute study in human healthy volunteers showed a significant rise in total antioxidant status at the end of 4 h after administration of 3 g root powder.

This study suggests that the plant possesses antioxidant activities which can counteract the oxidative damage. Total flavonoid contents in the extract was determined by using quercetin as standard. The total flavonoid contents of methanol extract of *Bombax ceiba* root was 13.816±1.06 mg/g equivalents of quercetin. Presence of flavonoid gives good indication of presence of antioxidant activity in *B.ceiba* root.

A report indicates that plant extracts (methanol and aqueous) were active against multi-drug resistant *Salmonella typhii* and *Klebsiellapneumoniae*. The present study indicates that methanol extract of *Bombax ceiba* root possesses mild to moderate antibacterial activity. The antimicrobial screening using a sensitive in-vitro discs diffusion method & the methanol extract was not good against all the test organisms. The methanol extract (300µg/disc & 600µg/disc) of *B.ceiba* root showed mild to moderate antibacterial activity against gram positive and gram negative bacteria.

The present study demonstrates that the methanol extract of *Bombax ceiba* root can be considered as a valuable source of therapeutic agents for human health, as an antioxidant, antimicrobial agent. The medicinal values of the *Bombax ceiba* root may be related to their constituent phytochemicals. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. Due to the antioxidant activity; it will be useful for the treatment of the diseases which are caused by oxidation

such as, neurodegenatation, asthma, anemia, ischemia etc. Since a variety of constituents is present in the extracts studied, it becomes difficult to describe the all properties selectively to any one group of constituents without further studies, which are beyond the scope of this paper. So further extensive investigation are necessary to find out and isolate potential constituents.

CHAPTER

FIVE

CONLUSION

5.1. Conclusion

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines.

Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectancy.

CHAPTER

SIX

REFERENCES

6.1. References

Ainsworth, E.A. and Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*. Vol.2(4), pp. 875-877.

Anandarajagopal, K., Sunilson, J.A., Ajaykumar, T.V., Ananth, R. and Kamal, S. (2013). In-vitro Anti-Inflammatory Evaluation of Crude *Bombax ceiba* Extracts. *European Journal of Medicinal Plants*. Vol.3(1), pp. 99-104.

Balunas, M. and Kinghorn, A. (2005). Drug discovery from medicinal plants. *Life Sciences*. Vol.78(5), pp. 431-441. [Online], Available at: http://www.sciencedirect.com/science/article/pii/S0024320505008799 [Accessed 29 Sept 2015].

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*. Vol.45(4), pp. 493-496.

Bhavsar, C.J. and Talele, G.S. (2013). Potential anti-diabetic activity of *Bombax ceiba*. *Bangladesh Journal of Pharmacology*. Vol.8, pp. 102-106.

Chakraborty, D.D., Ravi, V. and Chakraborty, P. (2010). Phytochemical evaluation and TLC protocol of various extracts of *Bombax ceiba* Linn. *International Journal of Pharmaceutical Sciences and Research.* Vol.1(8) : 0975-8232.

Chaudhary, P.H. and Khadabadi, S.S. (2012). *Bombax ceiba* Linn.: Pharmacognosy, Ethnobotany and Phyto-pharmacology. *Pharmacognosy Communications*. Vol.2(3), pp. 1-7.

Chaudhary, P.H., Rai, P.D., Deore, S.L. and Khadabadi, S.S. (2014). Pharmacognostical and phytochemical studies on roots of *Bombax ceiba* Linn. *Journal of Pharmacy & Pharmacognosy Research*. Vol.2 (6), pp. 172-182.

Chapter Six: References

Cleveland Clinic (2009). *Plant Sterols and Stanols*, Available at: https://my.clevelandclinic.org/health/diseases_conditions/hic_Cholesterol/hic_Plant_Sterols_and_Stanols [Accessed 2 Oct 2015].

Cui, J. , Guo, S. and Xiao, P. (2011). Antitumor and antimicrobial activities of endophytic fungi from medicinal parts of Aquilaria sinensis. *Journal of Zheijang University Science - Biomedicine & Biotechnology*. Vol.12(5), pp.385-392. [Online], Available at: http://www.ncbi.nlm.nih.gov/pubmed/21528493 [Accessed 17 Sept 2015].

Ghani, A. (1998). *Medicinal plants of Bangladesh : Chemical constituents and uses*. Asiatic Society of Bangladesh, Dhaka, Banglaesh.

Ghani, A. (2003). *Medicinal plants of bangladesh with chemical constituents and uses*. Asiatic Society of Bangladesh. 2nd ed.

Herbcyclopedia. (2014). *Bombax ceiba, the cotton tree*, Available at: http://www.herbcyclopedia.com/item/bombax-ceiba-the-cotton-tree-2 [Accessed 2 Oct 2015].

Hill, A. F. (1952). *Medicinal Plants: Drug Classification*, Available at: http://www.faculty.ucr.edu/~legneref/botany/medicine.htm [Accessed 6 Sept 2015].

Hodel, D.R. and Weissich, P.R (2012). Trees in the landscape, Part 4: *Bombax ceiba*. *Western Arborist*, Available at: http://ucanr.edu/sites/HodelPalmsTrees/files/186123.pdf [Accessed 2 Aug 2015].

Khan, M. S., Rahman, M. M. and Ali, M. A. (2001). *Red data book of vascular plants of Bangladesh. (eds)*. B.N.H, Dhaka, Bangladesh.

Linh, T. N., Joseph, I. O. and William, R. F. (2013). *21st Century Natural Product Research and Drug Development and Traditional Medicines*, [Online], Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3652390 [Accessed 5 Sept 2015].

Markley, J. (2015). *What Are Plant Steroids*, Available at: http://www.wisegeek.com/what-are-plant-steroids.htm [Accessed 2 Oct 2015].

Chapter Six: References

Medicinehunter. (2014). *About Plant Medicines | Medicine Hunter*, Available at: http://www.medicinehunter.com/about-plant-medicines [Accessed 6 Sep 2015].

Medknow Publications. (2012). *Historical review of medicinal plants' usage*, Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3358962 [Accessed 1 Sept 2015].

Mora, J.M., Mendez, V.V., & Gomez, L.D. (1999). White-nosed coati Nasua narica as a potential pollinator of Ochroma pyramidale. *Revista de Biologia Tropical*. Vol. 47(4), pp. 719-721.

Mpbd.info, (2014). *Medicinal Plants of Bangladesh*, Available at: http://www.mpbd.info/ [Accessed 13 Sept 2015].

Rajendra, K.C. (2008). *A brief introduction to Semal (Bombax ceiba Linn)*, Available at: http://www.forestrynepal.org/images/simal_present_report3.pdf [Accessed 5 Aug 2015].

Rameshwar, V., Kishor, D., Tushar, G., Siddharth, G. and Sudarshan, G. (2014). A Pharmacognostic and pharmacological overview on *Bombax ceiba*. *Scholars Academic Journal of Pharmacy*. Vol. 3(2), pp. 102-105.

Raskin, I., Ribnicky, D. and Komarnytsky, S. (2002). *Trends in Biotechnology*. 12th ed. [ebook] New Brunswick: Phytomedics Inc, vol.20, pp.522-530. [Online], Available at: http://www.researchgate.net/profile/Slavko_Komarnytsky2/publication/11024550_Plants_and_human_health_in_the_twenty-first_century/links/0912f50b39256431d4000000 [Accessed 6 Sept 2015].

Refaat, J., Desoky, S.Y., Ramadan, M.R., and Kamel, M.S. (2012). Bombacaceae: A phytochemical review. *Informa Healthcare USA, Inc.* ISSN 1744-5116, pp. 1-3.

Reiner, R. (1982). Antibiotics, an introduction. *New York: Thieme-Stratton*, pp.21-27.

Shashi, C., Abhishek, S., Chinu, and Shivani, C. (2015). A review on ethanobotanical and pharmacological uses of *Bombax Ceiba*. *Asian Pacific Journal of Pharmaceutical and Applied Sciences.* Vol.1(1), pp. 12-16.

Chapter Six: References

Taylor, L. (2000) *Plant Based Drugs and Medicines*, Available at: http://www.rain-tree.com/plantdrugs.htm#.VjUYtGJ96zd [Accessed 3 Sept 2015].

Verma, S. and Singh, S.P. (2008). Current and future status of herbal medicines. *Veterinary World*. Vol.1(11), pp. 347-350.

Watson, L. and Dallwitz, M.J. (1992). *Families of flowering plants: descriptions, illustrations, identification and information retrieval*, [Online], Available at: http://www.botany.hawaii.edu/faculty/carr/bombac.htm [Accessed 5 Oct 2015].

WHO. (2015). *Essential Medicines and Health Products Information,* Available at: http://apps.who.int/medicinedocs/en/d/Jh2945e/4.html [Accessed 2 Sep 2015].

Worldpress. (2005). *Importance of Medicinal plants,* Available at: https://ayuvedaherbs.wordpress.com [Accessed 1 Sept 2015].

Yusuf, M. , Wahab, M. , Chowdhury, J. and Begum, J. (2007). Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. *Bangladesh Journal of Plant Taxonomy*. Vol.14(2), pp.15-28. [Online], Available at: http://www.scribd.com/doc/4003274/Some-Common-Medicinal-Plants-of-Bangladesh-With-scientific-name-and-use [Accessed 12 Sept 2015].