East West University

Pharmacological Investigations of Roots of *Bombax ceiba*

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

Submitted By

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled **"Pharmacological Investigations of Roots of *Bombax ceiba*"** 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.

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"Pharmacological Investigations of Roots of *Bombax ceiba*" is an authentic research work done by **Mir Md Nakir Hossain**, ID: 2011-3-70-019 under the guidance of **Nazia Hoque**, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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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 **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 **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, Ishrat Jahan, Sumaiya Simin, and Faizul Hafiz for their endless cooperation and whole hearted 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.

Mir Md Nakir Hossain

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DEDICATION

THIS RESEARCH PAPER IS DEDICATED

TO MY BELOVED PARENTS,

WHO ARE MY BIGGEST INSPIRATIONS…

ABSTRACT

Plants have been an important source of medicines since the beginning of cultivation. There is a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Bombax ceiba* Linn (Family: Bombacaceae) is a tall tree used by various tribal communities and forest dwellers for the treatment of a variety of ailments. The plant literature survey shows the plant possesses astringent, cooling, stimulant, diuretic, aphrodisiac, demulcent, and tonic effects and also helps in dysentery. It also possesses important pharmacological activity such as aphrodisiac, anti-inflammatory and hepatoprotective activity in addition to anticancer and anti-HIV activity, anti-Helicobacter pylori, antiangiogenic, analgesic and antioxidant activity and hypotensive, hypoglycemic and antimicrobial activity. It is reported to contain important phytoconstituents such as naphthol, napthaquinone, polysaccharides, anthocyanins, shamimin and lupeol. These are the influencing information of this study.

The aim of the present study was to evaluate the antimicrobial, antioxidant activities and cytotoxic effects of the Dichloromethane root extract of *Bombax ceiba*.

The powdered root of *Bombax ceiba* was extracted with Dichloromethane. The concentrated crude Dichloromethane extract was then evaluated for antimicrobial, antioxidant and cytotoxic activities.

The antimicrobial activities of Dichloromethane crude extract of *Bombax ceiba* were tested against the *Shigella dysenteriae, Pseudomonas aureus,* and *Saccharomyces cerevisiae.* The antimicrobial test was performed by disc diffusion method. The concentrations used in this study were 300 µg/ disc and 600 µg/ disc. The range of zone of inhibition was 7-10 mm.

The *in vitro* antioxidant activity test was performed by Aluminium Chloride colorimetric method in which the flavonoid concentration was measured by using a standard curve of Quercetin. The flavonoid concentration found was 7.847 ± 0.649 mg/L.

The cytotoxicity was performed by using brine shrimp hatched in the laboratory. The different concentrations of sample was used: 400 µg/ ml, 200 µg/ ml, 100 µg/ ml, 50 µg/ ml, 25 µg/ ml, 12.5 µg/ ml, 6.25 µg/ ml, 3.125 µg/ ml, 1.5625 µg/ ml and a blank with both positive and negative controlled test. The test result shows the lethal effect. From the test result LC_{50} has been determined which are 19.85 ± 0.89418 µg/ ml.

Key words: *Bombax ceiba,* antimicrobial activities, antioxidant activities, cytotoxic effects, Dichloromethane root extract of *Bombax ceiba.*

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INTRODUCTION

1.0 General Introduction

Natural environment has been a source of medicinal agents for thousands of years, since healing with plants dates back probably to the evolution of *Homo sapiens*. Even to date, about 80% of the world's inhabitant's rely mainly on traditional medicines for their primary health care, while medicinal plants continue to play an important role in the health care systems of the remaining 20%. Partly based on their use in traditional medicine, an impressive number of modern drugs have also been isolated from natural plant species. Remarkably, even today there real definition for this special group of plants that has been accompanying mankind throughout history. Most frequently, medicinal plants are defined as feral and/or cultivated plants that, based on tradition and literature records, can be directly or indirectly used for medical purposes. The basis for this use is that these plants contain so called active ingredients (active principles or biologically active principles) that affect physiological (metabolic) processes of living organisms, including human beings. The notion of aromatic plants is even less definite. The attribute aromatic indicates plants having an aroma; being fragrant or sweet-smelling, while the word aroma is supposed to imply also the taste of the material (aromatic herbs). Spice plants are plants used for seasoning, spicing, flavoring and coloring foods, drinks and different products of the food processing industry, i.e. making a product more enjoyable. (Solecki and Shanidar 1975)

1.1 Overview on Medicinal Plants

Those plants that have healing properties are termed as medicinal plants or herbs. The plant kingdom is divided into several groups, but the botanical classification is beyond the scope of this section. However, medicinal plants can be simply classified as trees, shrubs, woody perennials, annuals and biennials, and climbers. (Dr. Everaldo G. Attard, 2005)

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs that humans have used throughout history to either cure or lessen symptoms from an illness. (Brekke Peterson Munks, 2012)

According to WHO, a medicinal plant is 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.

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found (Solecki and Shanidar 1975).

Since prehistoric times, shamans or medicine men and women of Eurasia and the Americas acquired a tremendous knowledge of medicinal plants. The fact that hundreds of additional species were also used by First Nations Canadians (Arnason et al. 1981) suggests that many of these also have important pharmacological constituents that could be valuable in modern medicine.

Up until the 18th century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16th century Italy, in Pisa, Padova and Florence, were medicinal plant gardens attached to medical faculties or schools. (Muller and Clauson, 1998)

The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke 1985). A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients. (Duke 1993)

According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements since they cannot afford the products of Western pharmaceutical industries together with their side effects and lack of healthcare facilities (Griggs et al., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu et al., 1999; Idu et al., 2007; Mann et al., 2008; Ammara et al., 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses. (Maheshwari et al., 1986; Van Wyk et al., 2000)

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell et al., 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many potent and powerful drugs. (Srivastava, et al., 1996; Mahesh and Sathish, 2008)

1.2 History of Medicinal Plants

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals. (Stojanoski Veles et al., 1999) In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis. (Kelly K et al. 2009)

1.2.1 Ancient Times

Fossil Records has revealed the use of medicinal plants by human beings around 60,000 years ago during Middle Paleolithic Age, (Fabricant & Farnsworth, 2001). These Fossil records suggest that even Neanderthal were not an exception who did not make use of medicinal plants, (Das and Choudhury, 2012).

Example of such medicinal plants is *Gingko biloba* which has been used medicinally for thousands of years. It is used for the treatment of numerous conditions such, many of which are under scientific investigation. The species has an evolutionary lineage that dates back to the Lower Jurassic, about 190 million years ago. Although this genus has undergone much change over this length of time, fossilized leaf material from the Tertiary species *Ginkgo adiantoides* is considered similar or even identical to that produced by modern *Ginkgo biloba* trees, (Jalalpour, et al 2012).

Figure 1.1: A Fossilized *Gingko adiantoides* Leaf similar to its modern day predecessor *Gingko biloba*

The Gingko plant is wide used in Alzheimer's disease, Cerebro vascular Insufficiency, and Cognitive Enhancement, Depression, Diabetes, Intermittent Claudication, Macular Depression, PMS, Sexual Dysfunction, Tinnitus, (Pelton, 2000).

In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium). In 1500 B.C., the Ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake. (Sumner, Judith, 2000)

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Earliest Sanskrit writings such as the Rig Veda, and AtharvaVeda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system.(Sumner, Judith, 2000) Many other herbs and minerals used in Ayurveda were

later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources. (Aggarwal, Sundaram, Malani, 2007; Dwivedi, 2007)

The mythological Chinese emperor Shennong is said to have written the first Chinese pharmacopoeia, the "Shennong Ben Cao Jing". The "Shennong Ben Cao Jing" lists 365 medicinal plants and their uses - including Ephedra (the shrub that introduced the drug ephedrine to modern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy). (Sumner, Judith, 2000)

The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains, scholars have noted that there is a large amount of overlap with the Egyptian herbals. (Robson, Barry & Baek, 2009)

Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (e.g. De herbis et curis) and - especially - Galen (e.g. Therapeutics), provided the pattern for later western medicine. Sometime between 50 and 68 A.D., a Greek physician known as Pedanius Dioscorides wrote "De Materia Medica", a compendium of more than 600 plants, 35 animal products, and ninety minerals. De Materia Medica remained the authoritative reference of herbalism into the 17th century. Similarly important for herbalists and botanists of later centuries was Theophrastus' "*Historia Plantarum*", written in the 4th century BC, which was the first systematization of the botanical world. (Grene, Marjorie, 2004)

1.2.2 Middle Age

Benedictine monasteries were the primary source of medical knowledge in Europe and England during the Early Middle Ages. Many Greek and Roman writings on medicine, as on other subjects, were preserved by hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. A 12th-century Benedictine nun, she wrote a medical text called *Causae et Curae*. (Elly et al. 2009)

Medical schools known as Bimaristan began to appear from the 9th century in the medieval Islamic world among Persians and Arabs, which was generally more advanced than medieval Europe at the time. Muslim botanists and Muslim physicians significantly expanded on the earlier knowledge of materia medica. The experimental scientific method was introduced into the field of materia medica in the 13th century by the Andalusian-Arab botanist Abu al-Abbas al-Nabati, the teacher of Ibn al-Baitar. This allowed the study of materia medica to evolve into the science of pharmacology. (Huff and Toby, 2003)

Avicenna's The Canon of Medicine lists 800 tested drugs, plants and minerals. Book was devoted to a discussion of the healing properties of herbs including nutmeg, senna, sandalwood, rhubarb, myrrh, cinnamon and rosewater. The Canon of Medicine remained a medical authority, used at many European and Arab medical schools, until the early 19th century. In particular, the Canon introduced clinical trials, randomized controlled trials, and efficacy tests. (Craig and Walter et al. 2000)

1.2.3 Early Modern Era

The 15th, 16th, and 17th centuries were the great age of herbals, many of them available for the first time in English and other languages rather than Latin or Greek. The first herbal to be published in English was the anonymous Grete Herball of 1526. The two best-known herbals in English were The Herball or General History of Plants (1597) by John Gerard and The English Physician Enlarged (1653) by Nicholas Culpeper. The Age of Exploration and the Columbian Exchange introduced new medicinal plants to Europe. Paracelsus introduced the use of active chemical drugs (like arsenic, copper sulfate, iron, mercury, and sulfur) in this era. (Kremers, Edward et al. 1986)

1.3 Traditional Medicine

Traditional medicine (also known as indigenous or folk medicine) comprises knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. (WHO, 2015)

In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called alternative medicine. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, and traditional African medicine. Core disciplines which study traditional medicine include herbalism, ethno medicine, ethno botany, and medical anthropology. (WHO, 2015)

Traditional medicine may include formalized aspects of folk medicine. Folk medicine consists of the healing practices and ideas of body physiology and health preservation known to some in a culture, and practiced or applied by anyone in the culture having prior experience. Folk medicine may also be referred to as traditional medicine, alternative medicine, indigenous medicine, or natural medicine. These terms are often considered interchangeable. (Acharya, et al. 2008)

There are two distinct forms of Traditional medicine practice. One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:

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

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

a) The Ayurvedic system which is the old Indian system and
b) The Unani system which has been developed by the Arab and Muslim scholars from the ancient Greek system.

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. (WHO, 2010)

1.3.1 Ayurveda

Ayurveda or Ayurvedic medicine is a system of Hindu traditional medicine native to the Indian subcontinent. Practices derived from Ayurvedic traditions are a type of alternative medicine. Ayurveda is a discipline of the upaveda or "auxiliary knowledge" in Vedic tradition. The origins of Ayurveda are also found in the AtharvaVeda, which contains 114 hymns and incantations described as magical cures for disease. There are also various legendary accounts of the origin of Ayurveda. Ayurvedic practices include the use of herbal medicines, mineral or metal supplementation (rasa shastra), surgical techniques, opium, and application of oil by massages. (Wells& John C, 2009)

Originated in prehistoric times, some of the concepts of Ayurveda have been discovered since the times of Indus Valley Civilization and earlier. Ayurveda significantly developed during the Vedic period and later some of the non-Vedic systems such as Buddhism and Jainism also incorporated in the system. Balance is emphasized, and suppressing natural urges is considered unhealthy and claimed to lead to illness. Ayurveda names three elemental substances, the doshas (called Vata, Pitta and Kapha), and states that a balance of the doshas results in health, while imbalance results in disease. Ayurveda has eight canonical components, which are derived from classical Sanskrit literature. Some of the oldest known Ayurvedic texts include the Sushruta Samhita and Charaka Samhita, which are written in Sanskrit. (Manohar, et al 2009)

Modern Ayurvedic medicine is considered pseudoscientific. Other researchers consider it a proto science, an unscientific, or trans-science system instead. Concerns were raised when 20% of Ayurvedic U.S. and Indian-manufactured patent medicines sold through the Internet were found to contain toxic levels of heavy metals such as lead, mercury, and arsenic. (Quack, et al. 2011)

Table 1.1: List of some Plants Used in Ayurveda

Species	Name	Uses in Ayurveda
Achillea millefolium	Biranjashipa	Carminative Tonic
Argyreia speciosa	Elephant creeper	Geriatric Tonic Mild Aphrodisiac
Asparagus recemosus	Wild Asparagus	Blood Purifier Rejuvanative
Capparis spinosa	Capers	Hepatic Stimulant
Cicorium intybus	Wild Chicory	Hepatic Stimulant
Commiphora mukul	Guggul	Antioxidant
Crocus sativus	Saffron	Hepatoprotective
Cyperus scariosus	Umbrella‟s Edge	Diuretic
Didymocarpus pedicellata	Shilapushpa	Cardiotonic
Garcinia camboia	Garcinia	Immunomodulator
Glycyrrhiza glabra	Licorice	Antioxidan Laxative
Gymnema sylvestre	Gurmara	Anti – diabetic

1.3.2 Unani

Unani-tibb or Unani Medicine also spelled Yunani Medicine is a form of traditional medicine practiced in countries of the Middle East and South Asia. It refers to a tradition of Graeco-Arabic medicine, which is based on the teachings of Greek physicians Hippocrates and Galen, and developed into an elaborate medical system in the Middle Ages by Arabian and Persian physicians, such as Rhazes (al-Razi), Avicenna (IbnSena), Al-Zahrawi, and Ibn Nafis.

Unani medicine is based on the concept of the four humours: Phlegm (Balgham), Blood (Dam), Yellow bile (Ṣafrā') and Black bile (Saudā'). The time of origin is thus dated at circa 1025 AD, when Avicenna wrote The Canon of Medicine in Persia. While he was primarily influenced by

Greek and Islamic medicine, he was also influenced by the Indian medical teachings of Sushruta and Charaka.

Unani medicine first arrived in India around 12th or 13th century with establishment of Delhi Sultanate (1206–1527) and Islamic rule over North India and subsequently flourished under Mughal Empire. Alauddin Khilji had several eminent Unani physicians (Hakims) in his royal courts. In the coming years this royal patronage meant development of Unani practice in India, but also of Unani literature with the aid of Indian Ayurvedic physicians. (Philosophy and Culture, New Delhi, 2001).

1.3.3 Traditional Chinese Medicine (TCM)

Traditional Chinese medicine is a broad range of medicine practices sharing common concepts which have been developed in China and are based on a tradition of more than 2,000 years, including various forms of herbal medicine, acupuncture, massage (Tuina), exercise (qigong), and dietary therapy. It is primarily used as a complementary alternative medicine approach. TCM is widely used in China and it is also used in the West. (Singh, et al. 2008)

TCM "holds that the body's vital energy circulates through channels, called meridians that have branches connected to bodily organs and functions." Concepts of the body and of disease used in TCM have notions of a superstitious pre-scientific culture, similar to European humoral theory.

The TCM theory and practice are not based upon scientific knowledge, and its own practitioners disagree widely on what diagnosis and treatments should be used for any given patient. The effectiveness of Chinese herbal medicine remains poorly researched and documented. There are concerns over a number of potentially toxic plants, animal parts, and mineral Chinese medicinal. Pharmaceutical research has explored the potential for creating new drugs from traditional remedies, but few successful results have been found. A Nature editorial described TCM as "fraught with pseudoscience", and said that the most obvious reason why it hasn't delivered many cures is that the majority of its treatments have no logical mechanism of action, yet proponents argue that it is because research has missed key features of the art of TCM, such as the interactions between different ingredients. (Shang, et al. 2007)

The doctrines of Chinese medicine are rooted in books such as the Yellow Emperor's Inner Canon and the Treatise on Cold Damage, as well as in cosmological notions such as yin-yang and the five phases. In the 1950s, the Chinese government promoted a systematized form of TCM. TCM's view of the body places little emphasis on anatomical structures, but is mainly concerned with the identification of functional entities (which regulate digestion, breathing, aging etc.). While health is perceived as harmonious interaction of these entities and the outside world, disease is interpreted as a disharmony in interaction. TCM diagnosis aims to trace symptoms to patterns of an underlying disharmony, by measuring the pulse, inspecting the tongue, skin, and eyes, and looking at the eating and sleeping habits of the person as well as many other things. (Steven Novella, 2012).

1.4 Medicinal Plant: Center of Research

Death is authentic but unavoidable. Nobody can desire to lose his short but sweet life. Man is therefore, being continued his struggle to achieve mastery over the forces of nature- Diseases Decay and Death. Human struggle against the misery of three D"s-Disease, Decay and Death is eternal. From the very inception of civilization, the inherent concern for getting as well as staying healthy has been instigating human venture for cure from his surroundings. Illness, physical discomforts, injuries, wounds & fear of death had forced prehistoric man to use any natural substances that he/she could lay his/her hands on- "the green friends" PLANTs (Ogdan, 1981).

The Plant kingdom consists of many different plant species containing different substances of medicinal importance. Some of these have already been explored for biological activity while some are not, (Rahman, *et al.* 2008).

As a source of medicine plant materials are important components of health care system. There are about 250,000 higher plant species (both Angiosperms and Gymnosperms) with a lower limit of 215,000 and upper limit of 500,000. Among these only 6% have been screened for biological activity and 15% have been evaluated phytochemically, (Fabricant & Farnsworth, 2001). Only just in South East Asia and its surrounding parts, there exist about 50,000 plant species among which 3,000 plants have been documented for potential medicinal properties and around 6,000 plants are used by traditional practitioners, (Shariff, et al. 2006).

So, Plants have been the traditional source of raw materials for medicine. It is known through the scholastic works of AtharvaVeda and the writings of Charaka and Sushruta which gave huge knowledge of preventive and curative medicinal to the scientific community, (Chowdhury, et al. 2008).

Now, nearly 95% of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates, (Maridass et al. 2008).

It has been estimated that about 13000 plant species around the world are used as drugs. Since, the inclination of using natural product has increased; the exploration of active plant extracts has become frequent for new drug discovery, (Ferdous, et al. 2010).

Over 50% of all advanced clinical drugs are made of natural products that play an important role in drug development programs of the pharmaceutical industry. There are hundreds of medicinal plants which have a long history of curative properties against various diseases. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the higher plant, (Razvy, et al. 2011).

So, for being cheap, relatively safe and easily available, medicinal plants and herbs embody the foundation of traditional medicinal practice all over the world. Representing an untapped and huge reservoir of drugs either known or novel in origin, the medicinal plants are center of research to find out novel lead compounds, (Ambikar, et al. 2010).

1.5 Increasing popularity of medicinal plants

The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for First Nations and immigrant populations, who have tended to retain ethnic medical practices.

In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. The reasons for this interest are varied, and include:

- **Low cost:** herbals are relatively inexpensive and the cost of pharmaceuticals to governments and individuals is rising
- **Drug resistance:** the need for alternative treatments for drug-resistant pathogens limitations of medicine: the existence of ailments without an effective pharmaceutical treatment.
- **Medicinal value:** laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants.
- **Cultural exchange:** expanding contact and growing respect for foreign cultures, including alternative systems of medicine.
- **Commercial value:** growing appreciation of trade and other commercial economic opportunities represented by medicinal plants

However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine (Duke 1993, Cox and Balick 1994).

1.6 Classification of Medicinal Plant:

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

Table 1.2: Classification of medicinal plant

Based on the active constituents	
Aromatic Herbs	Fennel, Ginger, garlic, Lemon grass
Nerving Herbs	Ginger, Catnip
Astringent Herbs	Peppermint, Red raspberry
Bitter Herbs	Aloe, cascara, Liquorices
Mucilaginous Herbs	Althea, Aloe, Burdock, Comfrey
Nutritive Herbs	Acerola, Apple

Based on plant part use	
Whole plant	Boerhaaviadiffusa
Root	Dasamula
Stem	Tinosporacordifolia
Bark	Saracaasoca
Leaf	Aloe vera
Flower	*Biophytum sensityvum, Mimuso pselenji*
Fruit	Solanum species
Seed	Daturastramonium

Based on habit	
Grasses	Cynodondactylon
Sedges	Cyperusrotundus
Herbs	Vernoniacineria
Shrubs	Solanum species
Climbers	Asparagus racemosus
Tress	Azadirachtaindica

Based on habitat	
Tropical	*Andrographis paniculata*
Sub-tropical	*Mentha arvensis*
Temperature	*Atropa belladona*

Based on Therapeutic value	
Antimalarial	*Cinchona officinalis, Artemisia annua*

Anticancer	*Catharanthus roseus, Taxus baccata*
Antiulcer	*Azadirachta indica, Glycyrrhiza glabra*
Antidiabetic	*Catharanthus roseus, Momordica charantia*
Anticholesterol	*Allium sativum*
Anti-inflammatory	*Curcuma domestica, Desmodium gangeticum*
Antiviral	*Acacia catechu*
Antibacterial	*Plumbago indica*
Antifungal	*Allium sativum*
Antiprotozoal	*Ailanthus* sp., *Cephaelisi pecacuanha*
Antidiarrhoeal	*Psidium gujava*, *Curcuma domestica*
Hypotensive	*Coleus forskohlii, Allium sativum*
Tranquilizing	*Rauwolfia serpentine*
Anesthetic	*Erythroxylum coca*
Spasmolytic	*Atropa belladona, Hyoscya musniger*
Diuretic	*Phyllanthus niruri, Centella asiatica*
Astringent	*Piper beetle, Abrus precatorius*
Anthelmintic	*Quisquali sindica, Punica granatum*
Cardio tonic	*Digitalis* sp., *Thevetia* sp.
Antiallergic	*Nandina domestica, Scutellaria baicalensis*
Hepatoprotective	*Andrographis paniculata*

1.7 Goals of Using Medicinal Plants as Therapeutic Agents

The goals of using plants as sources of therapeutic agents are –

- To isolate bioactive compounds for direct use as drugs, (E.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastine, Vincristine);
- To produce bioactive compounds of novel or known origin as lead compounds for semi synthesis to produce molecules of higher activity and / or lower toxicity, (E.g. Metformin, Nabilone, Oxycodone and other narcotic analgesics, Taxotere, Teniposide,

Verapamil, and Amiodarone, which are based on Galegine, 9 – tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin respectively);

- To use agents as pharmacologic tools (E.g. LSD, Mescaline, Yohimbine); and
- To use the whole plant or part of it as a herbal remedy, (E.g. Cranberry, Echinacea, Feverfew, Garlic, Ginkgo biloba). (Fabricant, et al. 2001)

1.8 Importance of Medicinal Plants as Drugs

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

Plants have provided much life – saving pharmaceutical agents so far. And, there is an intense ongoing documentation of ethno medical data and scientific research on medicinal plants by many developing countries. 14 of 35 in every 2000 drugs are either natural products or their derivatives. The plants that are not studied phytochemically can thus provide potential new leads for newer drug development. For example, Galegine from the herb *Galega officinalis* was the lead compound for the development of Metformin used in the treatment of type 2 diabetes. (Ahmed 2011)

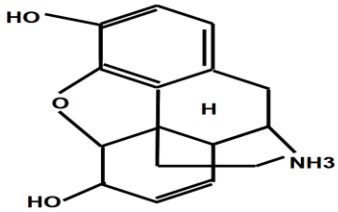
Table 1.3: Therapeutic Agents obtained from Flowering Plants

Plant Species	Therapeutic Agents
Atropa belladonna	Atropine
Camptotheca acuminate	Camptothecin
Catharanthus roseus	Vinblastine, Vincristin
Chondrodendron tomentosum	Tubocurarine
Digitalis lanata	Digitoxigenin, Gitoxigenin, Digoxigenin
Ephedra sinica	Ephedrine

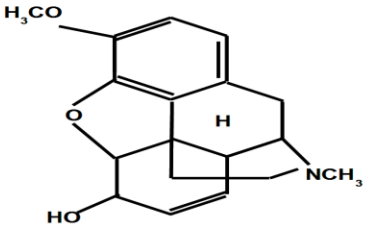
There are various types of chemical constituents found in plants having drug properties, healing properties and they have been used and still using for the preparation and a very important source for different potent drugs. Various classes are such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenes etc

1.8.1 Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste (Manske, 1965; Guillermo and Victor, 1999).



Morphine



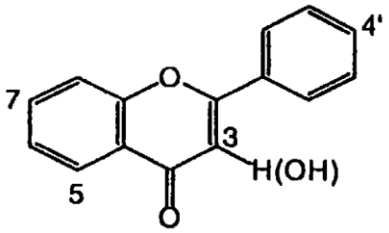
Codeine

Table 1.4: Methods for detection of alkaloids

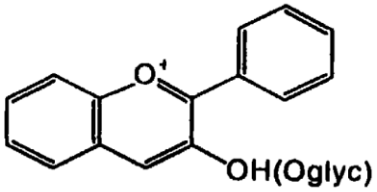
Reagent/ Test	Composition of the Reagent	Result
Meyer's reagent	Potassiomercuric iodide solution	Cream Precipitate
Wagner's reagent	Iodine in Potassium iodide	Reddish-Brown Precipitate
Tannic acid	Tannic acid	Precipitation
Hager's reagent	A saturated solution of picric acid	Yellow Precipitate
Dragendroff's reagent	Solution of potassium bismuth iodide potassium chlorate, a drop of HCl, evaporated to dryness and the resulting residue is exposed to ammonia vapor	Orange or reddish-brown Precipitate
Murexide test for caffeine	Murexide reagent	Purine alkaloids produce pink color

1.8.2 Flavonoids

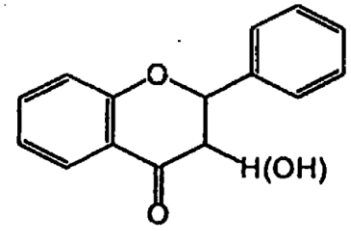
Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Rauha et al., 2000; Cushnie and Lamb, 2005; Filippos et al., 2007; Spencer and Jeremy, 2008).



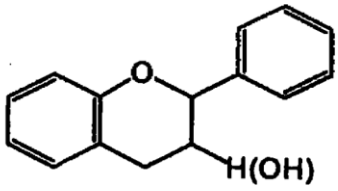
FLAVONE (FLAVONOL)



ANTHOCYANIDIN (ANTHOCYANIN)



DIHYDROFLAVONE (DIHYDROFLAVONOL)



FLAVAN (FLAVANOL)

1.8.3 Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom tri terpenes in plants. They are found in various plant parts; leaves, stems roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently (Oakenfull and Sidhu, 1990). Saponins also inhibit cancer tumor growth in animals,

particularly, lung and blood cancers, without killing normal cells. Saponins are the plant's immune system acting as an antibiotic to protect the plant against microbes and fungus (Shideler, 1980; Chatterrjee and Chakravorty, 1993).

1.8.4 Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative (Chatterrjee and Chakravorty, 1993; Samp, 2008)

1.8.5 Glycosides

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemiacetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colorless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phyto constituents, found in the cell sap (Kar, 2007; Firn, 2010). Glycosides are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action.

This group of drugs is usually administered in order to promote appetite and aid digestion. Glycosides are purely bitter which act on gustatory nerves, resulting in increased flow of saliva and gastric juices. (Sarker & Nahar, 2007). Glycosides have more different uses such as-

- Cardiac glycosides acts on the heart,
- Anthracene glycosides purgative, and for treatment of skin diseases
- Chalcone glycoside as anticancer agent,
- Amarogentin, gentiopicrin, andrographolide, ailanthone and polygalin are used as flavoring agents in many pharmaceutical preparations.

- Amygdalin has been used in the treatment of cancer, and also as a cough suppressant in various preparations.

1.8.6 Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos et al., 2007).

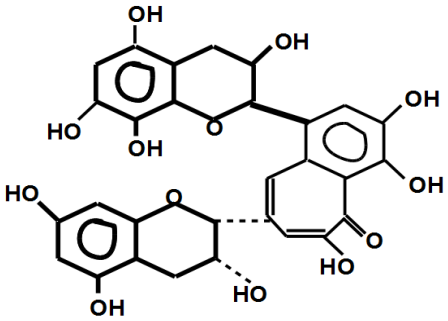
1.8.7 Phenolics

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural color pigments responsible for the color of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Puupponen- Pimiä *et al*., 2008). They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolies. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents.

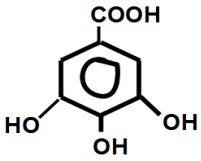
1.8.8 Tannins

These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolic or carboxylic group (Kar, 2007). They form complexes with proteins, carbohydrates, gelatin and alkaloids. Tannins are used as antiseptic and this activity is due to presence of the

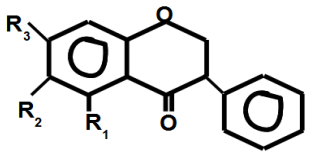
phenolic group. Common examples of tannins include theaflavins (from tea), daidezein, genistein and glycitein.



Theaflavin



Gallic acid



Daidzein: R_1=R_2=H; R_3=OH
Genistein: R_1=R_3=OH; R_3=H
Glycitein: R_1=H; R_2=OCH_3; R_3=OH

1.8.9 Terpenes

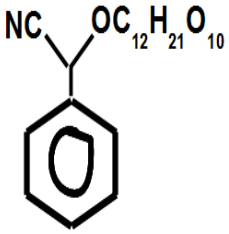
Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Firn, 2010). Examples of commonly important monterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C20) are classically considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes (C30) include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes are amyrins, ursolic acid and oleanic acid. Sesquiterpene (C15) is major components of many essential oils (Martinez et al., 2008). The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic action. The sesquiterpene lactone, palasonin, has anthelmintic activity, inhibits glucose uptake.

1.8.10 Essential Oils

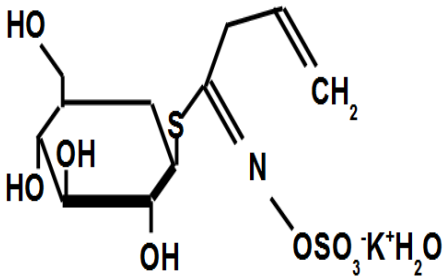
Essential oils are the odorous and volatile products of various plant and animal species. Essential oils have a tendency evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils. They mostly contribute to the odoriferous

constituents or 'essences' of the aromatic plants that are used abundantly in enhancing the aroma of some spices (Martinez et al., 2008).

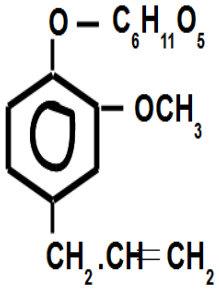
Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odor (Firn, 2010).



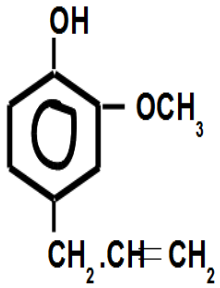
Amygdalin



Sinigrin



Gein



Eugenol

Table 1.5: Mechanism of action of some phytochemicals

Phytochemicals	Activity	Mechanism of Action
Quinones	Antimicrobial	Binds to adhesins, complex with cell wall, inactivates enzymes
Flavonoids	Antimicrobial Antidiarrhoeal	Complex with cell wall, binds to adhesins, Inhibits release of autacoids and prostaglandins, Inhibits contractions caused by spasmogens, Stimulates normalization of the deranged water transport across the mucosal cells, Inhibits GI release of acetylcholine.
Polyphenols & Tannins	Antimicrobial Antidiarrhoeal Anthelmintic	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation.

		Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action. Increases supply of digestible proteins by animals by forming protein complexes in rumen
Coumarins	Antiviral	Interaction with eukaryotic DNA
Terpenoids & Essential oils	Antimicrobial Antidiarrhoeal	Membrane disruption; Inhibits release of autacoids and prostaglandins
Alkaloids	Antimicrobial Antidiarrhoeal Anthelmintic	Intercalates into cell wall and DNA of parasites; Inhibits release of autacoids and prostaglandins; Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis
Lectins & Polypeptides	Antiviral	Blocks viral fusion or adsorption, forms disulfide bridges
Glycosides	Antidiarrhoeal	Inhibits release of autacoids and prostaglandins
Saponins	Antidiarrhoeal Anticancer Anthelmintic	Inhibits histamine release *in vitro*; Possesses membrane permeabilizing properties leads to vacuolization and disintegration of teguments
Steroids	Antidiarrhoeal	Enhance intestinal absorption of Na+ and water

1.9 Medicinal Plants: Natural Antibiotics

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.).

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink et al., 2005).

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy, 2009; Sathishkumar and Paulsamy, 2009).

In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weight are generally less than 2000.

A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed, 25% are plant derived (Farnsworth and Morris, 1976; Ogundipe et al., 1998). Plant compounds are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there is an increased attention on extracts and

biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro-organisms build against the antibiotics (Essawi and Srour, 1999). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants (Cox, 1994). Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medical practices (Gilani and Rahman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains. (Wink et al., 2005)

1.10 Medicinal Plants: Antimicrobial agents

Medicinal plants have always been considered as a source for healthy life for people. Therapeutic properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali et al., 1998; Barbour et al., 2004; Yasunaka et al., 2005).

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim, 1997; Towers et al., 2001; Koshy et al., 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Murray, 1992; Madunagu et al., 2001; Koshy et al., 2009; Senthilkumar and Reetha, 2009) Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases (Ikenebomeh and Metitiri, 1988; Rojas et al., 2003; Geyid et al., 2005).

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Paz et al., 1995; Vlietinck et al., 1995; Kudi et al., 1999; Palambo and Semple, 2001).

There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials (Barbour et al., 2004).

1.11 Medicinal Plants: Potential Antioxidants

In living systems, oxidation is a basic part of the normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated (Finkel and Holbrook, 2000; Halliwell, 2000; Pietta, 2000; Vijayabaskaran et al., 2010). Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging (Mclarty, 1997; Young and Wood, 2001; Yang et al., 2001; Sun et al., 2002; Bimal et al., 2011). Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body.

There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, (Zheng and Wang 2001; Cai et al., 2003; Govindarajan et al., 2005; Naruthapata and Supaporn, 2009). So to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems

associated with the above disease conditions (Halliwell, 1994; Klipstein et al., 2000; Bimal et al., 2011).

Naturally occurring antioxidants can be used in foods and also for prevention and treatment of free radical-related disorders (Middleton et al., 2000; Kumar and Kumar, 2009) which can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effects (Velioglu et al., 1998; Vinay et al., 2010). Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Shreejayan and Rao, 1997; Hagerman et al., 1998; Balakrishnan et al., 2009).

1.12 Medicinal Plants: Anti Cancer Activity

Cancer is one of the most life-threatening diseases with more than 100 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and side effects of anticancer drugs, cancer can be a cause of death. Cell death can occur through several different mechanisms, of which the most widely described are apoptosis and necrosis (Sengupta *et al*., 2004; George *et al*., 2010). A significant physiological consequence of cell death by apoptosis is that the apoptotic cells are immediately phagocytosed by macrophages. Therefore, the release of intracellular molecules that cause secondary disturbance to the surrounding tissue is limited to a low level compared with necrosis, which causes further tissue destruction and inflammation (Cohen, 1993; Earnshaw, 1995).

The importance of natural bioactive substances found in fruits, vegetables, and herbs, as antioxidants and functional foods are believed to be potential chemo preventive or therapeutic agents for cancer (Pezutto, 1997; Christou *et al.,* 2001; Mukherjee *et al.*, 2001). Most of these substances exert their chemotherapeutic activity by blocking the cell cycle progression and triggering apoptotic cell death. Therefore, the induction of apoptosis in tumor cells has become an indicator of the tumor-treating ability of naturally derived bioactive substances (Smets, 1994; Paschka *et al*., 1998).

The accepted modality for cancer treatment involves surgery, radiation and drugs, singly or in combination. Cancer chemotherapeutic agents can often provide temporary relief from symptoms, prolongation of life and occasionally leads to a cure. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells. The life span of both normal and cancer cells is significantly affected by the rate of apoptosis. Thus, modulating apoptosis may be useful in the management and therapy or prevention of cancer. Synthesis or modification of known drugs continues to be an important aspect of research.

Recent studies on tumor inhibitory compounds of plant origin have yielded an impressive array of novel structures. Epidemiological studies suggest that consumption of diets containing fruits and vegetables, which are major sources of photochemical and micronutrients, may reduce the risk of developing cancer. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells (Chiao *et al.,* 1995; Hirano *et al.,* 1995; Jiang *et al.,* 1996; Shaikh *et al.,* 2011).

Camptothecin has been effective against a broad spectrum of tumors. Camptothecin is a quinoline based alkaloid found in the bark of the Chinese camptotheca tree. It has been used for psoriasis, leukemia and diseases of liver, gall bladder, spleen and stomach.

Even though there are number of synthetic antitumor agents available, efforts are still on to search for effective naturally occurring anti carcinogens that would prevent, slow or reverse cancer development. Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds constitute more than 50% of anticancer agents (Newman *et al.,* 2003; Nipun *et al.,* 2011).

1.13 Medicinal Plants in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in "*Materiamedica*" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants, majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional

medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (Kritikarand Basu, 1980).

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

Table 1.6: List of some Medicinal Plants in Bangladesh

Scientific Name	Local Name	Traditional uses	Part(s) used
Bryonopsis laciniosa	Shivalingani	Skin Diseases, Dyspepsia, Jaundice	Whole Plant
Amorphophallus campanulatus	OlKachu	Piles, Tumors, Enlarged Spleen, Asthma, Rheumatism	Tuberous Roots
Hopea schaphula	Boilsur	Astringent, CNS depressant, Hypotensive	Stem Bark
Arachis hypogea	Cheenabadam	Emollient (Seeds), Bowel Astringent (Seeds Oil), Haemostatic Agent (Fruit Skin Extract)	Aerial Parts
Samanea saman	Fulkoroi	Diarrhea, Intestinal Diseases, Stomach Ache, Colds and Headache, Sore Throat	Bark
Michelia champaca	Champa	Fever, Colic, Leprosy, Post-Partum Protection, Eye Disorder	Seed and Flower

Aloe indica	Ghritakumari	Arthritis, hypertension Diabetes mellitus	Skin of Leaves
Swietenia mahagony	Mahagony	Diabetes, Malaria, Fever, Hypertension	Seeds
Caesalpinia nuga	Krung – khai	Analgesic, Anti Amyloidogenic, Antidiabetic, Hypoglycemic, Antifilarial, Anti- Inflammatory, Antimalarial, Antioxidant, Antitumor, Anxiolytic, Immunomodulatory	Seed
Adansonia digitata	Baobab, Gadhagachh	Anti malarial, Anti pyretic, Anti ulcerant, Health tonic	Leaf, Root, Flower
Jatropha gossypifolia	Karachuni, Bellyache Tree	Analgesic in toothache, Anti diarrhoeal, Anti malarial,	Leaf
Rauwolfia serpentine	Sharpagandha	Anti hypertensive, Anti malarial, Anti psychotic	Root
Hodgsonia macrocarpa	Makal	Anti malarial, Anti pyretic	Fruit

1.13.1. Tribal medicinal plant in Bangladesh

In different localities of Rangamati and Bandarban Districts of Bangladesh a survey was carried out between 2001 and 2002 to document medicinal plants. A total of 69 medicinal plants under 40 families were documented during this work, which the tribal use to treat about 50 diseases. (Yusuf et al.2006)

Table 1.7: Plants used by the Kavirajes and tribal medicinal practitioners of Bangladesh

Scientific name	Local name(s)	Plant part(s) used	Treatment
Callicarpa japanica	Rakabbory	Leaf	Dyspepsia, heart burn
Callicarpama crophylla	Jama-thoi	Whole plant	Tonic, dermatitis, cancer, antidote.
Clerodendrum indicum	Brahmonhati	Whole plant	rheumatoid arthritis, jaundice, skin diseases, edema, sedative
Clerodendrum inerme	Vana-jhai	Leaf, flower	Night blindness, pneumonia, colic, rheumatoid arthritis.
Clerodendrum trichotomum	Chapa-genda	Leaf, stem, flower	Heart diseases, rheumatoid arthritis, skin diseases.
Clerodendrum viscosum	Viti	Whole plant, leaf	Giddiness, typhus, colic in cattle, diabetes, fever, cold, aphrodisiac,
Duranta repens	Kata mehandi	Whole plant, fruit, bark	Insect repellent, itches, infertility, fever, pneumonia.
Lantana camara	Chaturaangi	Root, flower	Cough, mental diseases, fever.
Lippia alba	Khuria	Leaf	Cuts and wounds.
Lippianodi flora	Bhumi-okra	Leaf, stem, bark	Constipation, eczema, stroke, gonorrhea.

Nyctanthe sarbor	Shefali	Whole plant,	Influenza, hypertension,
Premnainte grifolia	Goniari	Leaf, bark, root	Fever, energy stimulant
Stachytarpheta indica	Supang	Leaf, stem	Leucorrhea.

1.13.2. Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (**IUCN, 2003**). The number of medicinal plants included in "***Materiamedica***" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants, majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (KritikarandBasu, 1980).

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

1.14 Research on Herbal Drug

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

- To increase efficacy of the drug.
- To remove toxic effects.
- To reduce side-effects.
- To maintain stability.
- To keep pleasant taste, color and odor.

1.14.1 Scientific Basis of Herbal Drug

Herbal drug is often criticized as non-scientific, inactive and erroneous medicine. But phytochemical and biological investigation proves its medicinal value and therapeutic utility. Traditional medicines that are used topically to treat skin disease contain tannin. Tannin is chemical having antiseptic and astringent property. When it is used topically it reacts with the proteins on infected area to produce a thin but strong barrier. This layer protects the infected area from micro-organism. Besides, tannin has antibiotic property. So it is said that there is no basic difference between herbal drug and allopathic medicine.

1.14.2 Rationale of Herbal Drug Research: Special Reference to Bangladesh

Most of the people of our country have no or little access to allopathic medicine due to their uncompromisable low income in respect of high cost of allopathic medicine. A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% if people suffering illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mollahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in a crude and substandard form, by our people. The use of such crude and substandard herbal drug is dangerous and may threaten public health. Thus the analysis of plants for exploring the bounty of chemical entities and their biological screening is the current need for standardization of herbal medication (Ghani, 1998). Since Bangladesh is a country of low economic growth, a proper health care system can be established by supplying low cost medicines to its population. This may be only possible by utilizing our natural resources of medicinal plants and their constituents. So, scientific exploration and standardization of these potential crude drugs is an urgent need to revolutionize our drug sector.

Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. During the last five years Bangladesh has spent more than 1500 crore Taka for importing chemicals, raw materials and semi-processed drugs of plant origin from neighboring and other countries and this trend is growing upwards day by day. This huge foreign exchange can be saved if the indigenous medicinal plants or its semi processed products are utilized by the manufacturer to satisfy their need (Ghani, 1998).

1.15 Natural Sources: A Model for Synthetic Drugs

Natural sources are contributing to the development of modern synthetic drugs and medicines in a number of ways as stated below (Ghani, 1998):

- Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.
- Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.
- Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs.

Though most of the modern medicines are gift of synthetic chemistry, there are still some synthetic drugs where plant constituents act as “lead” (precursor) molecule. Procaine, a synthetic compound, displaces cocaine, isolated from coca leaves, due to its lacking of addiction property. Due to relatively low therapeutic index of procaine, search of new synthetic products lead to synthesis of Lidocaine, tetracaine and dibucaine. The discovery of diosgenin from Mexican Yams (Dioscoria) as a starting material for the synthesis of progesterone decreases the cost of progesterone from 80 U.S. $ per gm to 1.7 U.S. $ per gm. Also lifesaving antibiotic penicillin is synthesized from a natural product 6-aminopenicillanic acid derived from *Penicillium notatum* (Goldstein, *et al*., 1974).

1.15.1 Necessity of Drug Development from Plant Sources

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role. Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents. So the application of herbal drug in crude form may be ineffective or may cause a toxic reaction. Many effective drugs has been developed from the crude extract of plants which is motivating many scientists to find out different therapeutic compounds and make a formula and synthetic route to develop a new a new drug against challenging diseases now a days. Some examples are given below:

- **Vincristine**, a prominent anticancer drug, was developed from periwinkle plant (*Vincarosea*) which was formerly prescribed for treating diabetes.
- The efficient hypotensive drug, **Reserpine**, was developed from *Rauwolfia serpentine* which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN *et al*., 1982).
- **Khelin**, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammi visnaga* which was formerly used as a diuretic and antispasmodic in renal colic.

Thus drug development from medicinal plants gives effective result (Ghani, 1998).

1.15.2 Procedure for Development

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

- Selection and correct identification of the proper medicinal plant.
- Extraction with suitable solvent(s).
- Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.

- Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.
- Repeated fractionation of active fractions to isolate pure compound(s).
- Elucidation of chemical structure of pure compound(s) using spectroscopic methods.
- Evaluation of biological activity of pure compound(s)
- Toxicological tests with pure compound(s).
- Production of drug in appropriate dosage forms.

1.15.3 Bioactivity Guided Research of Medicinal Plants

However, natural products are currently undergoing a phase of reduced attention in drug discovery because of the enormous effort which is necessary to isolate the active principles and to elucidate their structures (Grabley, *et al*. 1999). Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, information from traditional medicine, field observations or even random collection. One main strategy in the isolation of new leads consists of the so-called Bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. Bioactivity guided phytochemical approach, has three phases of investigation.

A. First, biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.

B. Second, the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained.

C. Third, the chemical structures of pure compounds are determined.

Only the bioactive extracts or fractions would be of connotation for next phytochemical and pharmacological analysis. So in medicinal plants research, bioactivity guided phytochemical approach might be a rational approach.

1.16 Plant Review

Scientific Name: *Bombax ceiba*

Bombax ceiba, like other trees of the genus *Bombax*, is commonly known as cotton tree. More specifically, it is sometimes known as red silk-cotton; red cotton tree; or ambiguously as silk-cotton or kapok. (U. Aguoru, 2015)

The Silk Cotton Tree is often referred to as the 'silent doctor' for the host of medicinal benefits that it offers. Each part of the tree, including the bark, flowers and leaves have therapeutic uses. A herbal composition made from the bark of the tree, for example, is administered for the treatment of sexual and gastrointestinal disorders. The tree is commonly found in India, Burma and Sri Lanka. (Brown, Stephen H, 2011)

This Asian tropical tree has a straight tall tree and its leaves are deciduous in winter. Red flowers with 5 petals appear in the spring before the new foliage. It produces a capsule which, when ripe, contains white fibers like cotton. Its trunk bears spikes to deter attacks by animals. Although it's stout trunk suggests that it is useful for timber, its wood is too soft to be very useful. (Brown, Stephen H, 2011)

1.16.1 Morphology

Fig 1.2: Bark with thorns

Fig 1.3: Bark

Fig 1.4: Palmate Leaf

The young stem and branches are covered with sharp, straight, stout prickles up to 1.2 cm long with woody conical bases.

Bark:

Bark of Semal 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. (Adrian and Jimmy Storrs, 1990)

Leaves:

Semal 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. (Adrian and Jimmy Storrs, 1990)

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.

The flowers of Semal 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. (Adrian and Jimmy Storrs, 1990)

Fig 1.5: Bisexual Flower

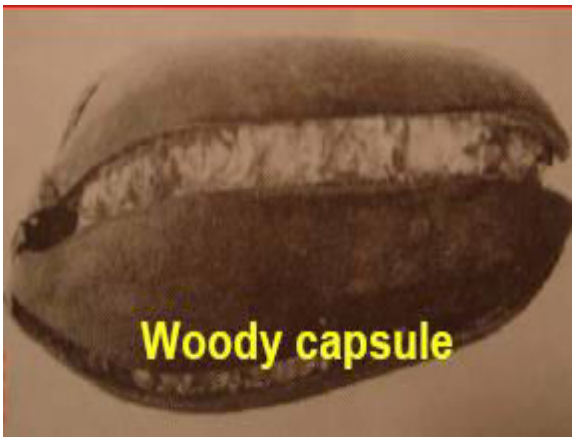


Fig 1.6: Woody Capsule

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. (Adrian and Jimmy Storrs, 1990)

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. (Adrian and Jimmy Storrs, 1990)

Fig 1.7: Seeds of *Bombax ceiba*

1.16.2 GENERAL DISTRIBUTION:

Bombax ceiba is widely distributed in Indian subcontinent except extremely arid regions ascending up to 1200 meters and occasionally up to 1500 meters. In Nepal, it is found from Terai (70 m) up to about 1300 meters.

It seeks moist, protected valleys preferably flat ground near stream banks where it is often gregarious. Though typical of the alluvial Savanna type of forests, it also grows sporadically in mixed deciduous forests in the lower valleys and in the Sal (*Shorea robusta*) forest. Though it is generally scarce in the hills, it is very common in the Bhabar and Terai tracts (tropics) of Nepal and India especially in the open grazing grounds in miscellaneous forests. It is often found growing in association with Sal (*Shorea robusta*).

Although it has a very wide range of distribution, it is nowhere very common, usually occurring scattered in mixed deciduous forests. Occasionally, it tends to be gregarious on alluvial soils near river banks and grassy savanna lands. It also occurs in Bangladesh, India, Sri Lanka, Pakistan, Myanmar, Java, Sumatra and Northern Australia. (Brown, Stephen H, 2011)

1.16.3 Growth Habit:

Bombax ceiba is called Kings of the Forest due to their massive size and showy flowers. It is a large deciduous tree with a straight cylindrical stem and horizontally spreading branches in whorls. This horizontally branching system in whorls, large size and the buttress at the base are the first seen characteristics to distinguish the species in the forest. The tree reaches up to 40 meter in height and 2 meter in diameter with the clear bole of 24-30 meter. Large trees are invariably buttressed at the base. Stem buttresses at the base and go up to 5-6 meter in height. (Smith, Mori, 2004)

Figure 1.8: A full view of *Bombax ceiba* Plant

Table 1.8: Vernacular Names of *Bombax ceiba*

Bangla	Shimul, Roktosimul, Katseori
English	Silk cotton tree, Kapok tree, Red cotton tree, Indian kapok
Hindi	Kaantisenbal, kantisembal, Rakat senbai, Semar kanda, Semul, Semur, Shembal, Shimbal, Simal, Simul, Shalmali, Semal
Sanskrit	Shalmali, semul, simul
Manipuri	Tera
Assamese	Dumboil, Himila, Himolu
Tamil	Sittan, Sanmali, Ilavam, Ilavu, Puula, Mullilavu
Malayalam	Unnamurika
Urdu	Sumbal
Marma	Lakh pine
Tanchangya	Chamful Gaith
Punjabi	Sumbal
Gujrati	Shimalo
Telgu	Buruga, KondaBuruga
Sinhala	Katu Imbul
Chinese	Hong mian, Ban zhi mian, Ying xiong shu
Burmese	Didu, Lepanbin, Letpan
Dutch	Simalboom, Randoe alas (as G. heptaphylla), Zijdekapokboom
French	Arbre *Bombax*, Fromager
German	Indischer Seidenwollbaum, Semul, Roter Seidenwollbaum
Greek	Vomvax malavarikos
Khmer	Roka.
Nepalese	Simal
Portuguese	Algodoeiro do mato, Bómbax, Bonga, Borracha, Borracho, Cartageno, Ceiba, Imbiruçu, Kapok, Paineira da India, Panha, Panheira, Sumaúma.
Spanish	Arbol capoc, Arbol kapok.
Thai	Ngio, Ngio ban, Ngio daeng, Ngio pong, Ngio pong daeng.

(Plants.usda.gov, 2014); (Tiwari, *et al*. 2000)

Table 1.9: Taxonomy of *Bombax ceiba*

Rank	Scientific Name & Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular Plants
Super Division	Spermatophyta – Seed Plants
Division	Magnoliophyta – Flowering Plants
Class	Magnoilopsida – Dicotyledons
Subclass	Dilleniidae
Order	Malvales
Family	Bombacaceae
Genus	*Bombax* L. – Cotton Tree
Species	*Bombax ceiba* L. – Red silk cotton tree

(Plants.usda.gov, 2014)

Table 1.10: Uses of various parts of *Bombax ceiba*

Various parts of *Bombax ceiba*	Uses of Parts
Bark	Emetic; used as a styptic in metrorrhagia
Gum	Used as acrid, astringent, demulcent, tonic, alterative, haemostatic and aphrodisiac; useful in diarrhea, dysentery, menorrhagia, cough, leucorrhoea, stomatitis and burning of the body.
Root	Has stimulant, demulcent, tonic, diuretic, emetic and aphrodisiac properties; given in impotence, leucorrhoea, over bleeding in menstruation, improve breast milk, cure cold & cough when used with black pepper and dry ginger powder
Young tap root	Astringent, and is used in dysentery.

Root powder	
Flowers	Used externally for boils, sores and itch
Aqueous extract of Stem Bark	Antibacterial and antifungal activity
Tender roots	To cure seminal disorders
Leaf	Used for blood purification
Thorny part of stem	Lightens scar marks due to boils, freckles, acne vulgarize and burns

(Ansari *et al.*, 2007)

1.16.4 Some Traditional uses of *Bombax ceiba*

- **Anti- helicobacter pylori properties:** Among several Taiwanese folk medicinal plants studied for their anti-helicobacter pylori properties only *Paederia scandens, Plumbago zeylanica, Anisomeles indica, Bombax ceiba* and *Alpinia speciosa* demonstrated anti-helicobacter pylori properties. (Wang and Huang, 2005)
- **Anti- Ageing properties:** Some studies suggested the potential use of *Bombax ceiba* for the prevention, reversal or delay of age-related diseases due to the antioxidant effect exerted by this herb. (Ngwuluka, 2012)
- **Anthelmintic, Vermifuge and Vermicides properties:** In some cases *Bombax ceiba* leaves exerted anthelmintic effects, a property that some plants and medicines have to help the body expel helminthes or parasitic worms. This use was reported at least in the traditional system of medicine in Southern Punjab of Pakistan, where it was able to fight live parasites (as trematode: *Paramphistomum explanatum*), collected from buffalo. (Hossain et al., 2011)
- **Inflammatory Bowel Disease:** As part of poly-herbal preparations against inflammatory bowel disease in rats, the aqueous extract of *Bombax ceiba* showed a certain beneficial effects, suggesting this plant has a protective role on inflammatory bowel disease cases. (Jagtap, Shirke and Phadke, 2004)
- **Anti-Microbial Properties:** *Bombax ceiba* also exhibited significant antioxidant and antimicrobial activities in other studies done on this plant.

- **Antioxidant Properties:** *Bombax ceiba* (Bombacaceae) has been used in traditional Chinese herbal medicine for the treatment of inflammatory conditions, diarrhea, fever, chronic inflammation, catarrhal affection, and as a diuretic. (Yu et al., 2011). The antioxidant activities of extracts from *Bombax ceiba* flowers exerted radical-scavenging activity, oxygen radical absorbency capacity (ORAC) and all the extracts possessed remarkable antioxidant capacity compared with ascorbic or gallic acids. The flowers of *Bombax ceiba* have excellent antioxidant properties and could be the source of natural antioxidants. (El-Hagrassi et al., 2011); (Yu et al., 2011)

1.16.5 Chemical Constituents of *Bombax ceiba*:

- All parts of the plant gave beta-sitosterol and its glucosides;
- Seeds, bark and root bark mainly contain lupeol;
- Flowers contain hentriacontane, hentriacontanol;
- Root bark, in addition, gave -hydroxycadalene.
- The seed oil yields arachidic, linoleic, myristic, oleic and palmitic acids;
- Seeds contain carotenes, n-hexacosanol, ethylgallate and tocopherols;
- The gum contains Gallic and tannic acids, yields L-arbinose, D-galactose, D-galacturonic acid and D-galactopyranose.
- Younger roots contain more sugars such as arbinose and galactose and peptic substances than the older ones. They contain mucilage, starch, mineral matter, tannins and non-tannins, along with other constituents.
- *Bombax ceiba* contains 7 important flavones- Vicenin, linarin, saponarin, cosmetin, isovitexin, xanthomicrol & apigenin.
- Other chemical constituents are found in very less amount such as steroids, triterpinoids, saponins, cholesterol, stigmasterol, campesterol, amyrins, cadinane sesquiterpenoids, isohemigossypol-1-methyl ester, acid lactone, napthaquinone, xanthones, and anthocyanins and so on. (Asolkar et al., 1992); (Reddy et al., 2003); (Niranjan and Gupta, 1973)

1.17 Plant material

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 h. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties. (Das.K, *et al.* 2010).

1.18 Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of

residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted.

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

1. **Water:** Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolic only important as antioxidant compound. (Das K *et al.* 2010).
2. **Acetone:** Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractants, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other Phenolics was better in aqueous acetone than in aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity. (Eloff JN, 1998)
3. **Alcohol**: The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have nonpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than

ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results. (Lapornik B*et al.*2005).

4. **Chloroform:** Terpenoids lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and Terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents.(Cowan MM, 1999)
5. **Ether:** Ether is commonly used selectively for the extraction of Coumarins and fatty acids.(Cowan MM, 1999)
6. **Dichloromethane:** It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only Terpenoids. (Cowan MM, 1999)

Table-1.11: Structural features and activities of various phytochemicals from plants

Phytochemicals	Structural features	Examples	Activities
Phenols & Polyphenols	C_3 side chain, - OH groups, Phenol ring	Catechol, Epicatechin, Cinnamic Acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketones substitutions	Hypericin	Antimicrobial
Flavonoids	Phenolic structure, one carbonyl group. Hydroxylated phenols, C_6-C_3 unit linked to an aromatic ring. Flavones + 3 hydroxyl group	Chrysin, Quercetin, Rutin	Antimicrobial Antidiarrhoeal

Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Saponins	Amphipathic glycosides	Vina-ginsenosides-R5 and -R6	Antidiarrhoeal
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching an cyclized	Capsaicin	Antimicrobial, Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and Polypeptides	Proteins	Mannose-specific agglutinin, Fabatin	Antimicrobial
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Coumarins	Phenols made of fused benzene and α-pyrone rings	Warfarin	Antimicrobial

(Maniyar.Y, *et al.*2010).

1.19 Methods of extraction

Variation in extraction methods usually depends upon:

- Length of the extraction period,
- Solvent used,
- pH of the solvent,
- Temperature

- Particle size of the plant tissues
- The solvent-to-sample ratio

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal. (Das K *et al.* 2010).

1.20 Antioxidants 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 thiol, 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 oxygen reactive 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.

1.20.1 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 bioflavonoid, 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.

1.20.2 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:

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.

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.

1.21 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:

I) 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 (Reiner, 1982). The primary assay can be performed *in vitro* by disk diffusion assay method, which includes

- ✓ Plate diffusion test
- ✓ 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.

ii) 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, 1982)

1.22 General Description: Bombacaceae Family

All of the Bombacaceae are trees, and most of them are large emergents with columnar trunks (i.e., not buttressed) and flat, spreading branches. Some have spiny trunks. The leaves are 3-veined (i.e., with three palmate veins at the base), palmately lobed, or palmately compound. The 3-veined genera are difficult to separate from Malvaceae by vegetative characters. Fruit or flowers may be necessary for a positive identification. Bombacaceae almost all have large, white, night-blooming flowers that are pollinated by bats. There are a large number of genuses and species present in this family. Among them, 9 species are most available and have medicinal use that are listed below with short description and some uses along with images of the plants.

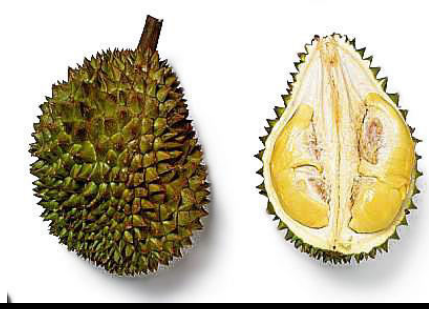
Table 1.12: Bombacaceae Family

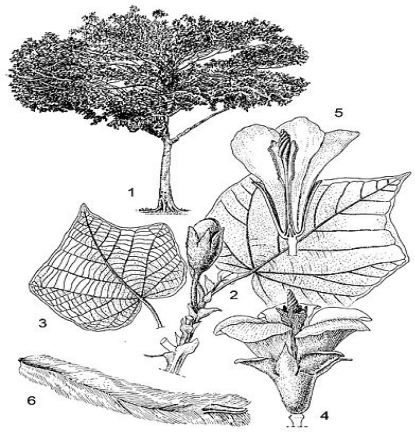
Images	Description	Uses
	***Adansonia digitata***, baobab, dead rat tree. Tree from tropical Africa with palmately lobed leaves and much enlarged trunk. The Kenyans say the devil planted this tree upside down because of the monstrous appearance with its swollen, bottle-shaped trunk and short dumpy branches sticking up in the air like thick roots. A trunk circumference of 62 feet has been reported in this species. Several trees in Africa are reckoned to be about 5,000 years old. The fragrant white flowers are about 6	Diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing, asthma, Malaria, fever, Toothache, gingivitis, Diaphoretic, fever remedy, Diaphoretic, kidney and bladder diseases, asthma, insect bites, Fever, diarrhoea, Microbial diseases, Anemia, Coughs, Wound healing

	inches across and are pollinated by bats. The 6 - 18 inch long fuzzy fruits are on long stalks giving the appearance of a hanging dead rat.	
	***Bombax ceiba***, red silk cotton tree. Large tree from tropical Asia with prickly trunk and palmate leaves bearing 3-7 leaflets. The common name refers to hairs (similar to but inferior to kapok) associated with the seeds.	Seminal disorders, nocturnal emission, blood purification, Leucorrhoea, over bleeding menstruation, acne, skin blemish, pigmentation, wounds, weakness, improve breast milk, cold and cough
	***Bombax glabra***, Guiana chestnut, Malabar chestnut. Tree from tropical S America. The fruit is woody, 4 - 12 inches long, and contains rounded seeds that are edible raw or roasted.	The seeds are used to prepare various delicious foods. Young leaves and flowers are also edible. The bark is used to treat stomach

		problems and headaches, and is taken to fortify the blood.
	***Ceiba pentandra*** is a tropical tree of the Bombacaceae family, native to Mexico, Central America, and West Africa. Kapok is the most used common name for the tree and may also refer to the cotton-like fluff obtained from its seed pods. The tree is cultivated for the seed fiber, particularly in south-east Asia, and is also known as the Java cotton, Java Kapok, silk cotton, Sumaúma or ceiba.	Antifungal, Antidiarrhoeal, Antiulcer, Hepatoprotective, Anthelmintic, Angiogenesis, Anti-inflammatory, Hypoglycemic, Hypolipidemic activity
	***Ceiba samauma***, lupuna. The lupuna tree is found throughout the Amazon. It is one of the giants of the rainforest, it is big, it is imposing, and it is well rooted in the jungle floor. It has a trunk	Blood purification, Leucorrhea, over bleeding menstruation, acne, skin blemish,

	that can be up to 33 feet across. The lupuna tree has a most unusual distinguishing mark which sets it apart from other forest trees. On the trunk, at its widest part there is a part that sticks out which resembles the shape of a human stomach.	Anthelmintic, Angiogenesis, Anti inflammatory, Hypoglycemic, Hypolipidemic activity
 	***Ceiba speciosa*** or the silk floss tree is a species of deciduous tree native to the tropical and subtropical forests of South America. It has a local name such as Palo Borracho (drunken stick).	The wood of the Ceiba speciosa tree is light, soft and flexible. It is used for packaging, making canoes and making paper and ropes. Oil from the seeds is used both as edible vegetable oil and for industrial applications. Silk floss trees are primarily used for ornamental purposes.

 	***Durio zibethinus***, durian. This is one of the most highly prized of tropical fruits, at least in Indonesia where it is used to flavor a variety of foodstuffs, including ice cream. Presumably it is an acquired taste as it has a very heavy odor that is disagreeable to most who encounter it for the first time.	*Durio zibethinus* is primarily used as a food source which is high in carbohydrates, potassium, and vitamin C. The fruit of the *D. zibethinus* is described as having a slight onion tang with flavors of caramel, vanilla, and banana. Fruits are sold as an aphrodisiac by the Surinam Chinese at Lelydorp.
	***Ochroma pyramidale***. It is commonly known as Balsa tree, a species of flowering plant. It is large, fast growing tree that can grow up to 30 m (98 ft) tall. Balsa wood is a very lightweight material with many uses. Balsa trees are native to Southern Brazil and Bolivia north to Southern Mexico.	The seeds fluffy kapok coat make it great for filling pillows and furniture. Balsa is valued for its soft, lightweight wood. It is ideal for rafts, boats, boxes, insulation, and model airplanes. The root or bark can be prepared and used as a diuretic. A flower decoction is used to treat colds and coughs.

		A decoction of flower and bark is can be used as an emetic. The sap from young balsa trees can be used as an external emollient. Some claim Balsa seeds have an edible oil.
 	***PseudoBombax ellipticum***, *Bombax*. Tree from tropical America with showy flowers having conspicuous pink or white stamens, often flowering when the tree is bare.	Seeds of the white-flowered form are eaten as a snack food. A decoction of the bark is a domestic remedy for coughs and catarrh and a decoction of the bark and root is used for treating toothaches and hardening of the gums. The cotton-like fiber within the pods is utilized for stuffing cushions and pillows. The calyx is utilized by the Indians of Sololá to make unique tobacco pipes. The wood is very brittle when freshly cut, but when seasoned it is satisfactory for fuel.

(Watson and Dallwitz, 2011)

1.23 Objectives

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

Table 1.13: Plant- *Bombax ceiba* (DICHLOROMETHANE extract)

SL No.	Experiments
01	Antimicrobial Activity Test
02	Antioxidant Activity Test
03	Cytotoxicity Test

The overall purpose and objective of the study is to analyze phytochemical substances present in the plant and evaluate the biological activities of *Bombax ceiba.*

1.24 Study area

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

1.25 Data collection

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

- **Primary sources**: direct personal contact and observations of the experiments carried out in the laboratory.
- **Secondary sources**: various publications like journals, papers, documents and websites.

1.26 Research protocol

- Selection, identification, collection, drying and grinding of plants.
- Extraction of the powders with methanol and DICHLOROMETHANE and collection of extract.
- Antioxidant activity determination.
- Anti-Microbial activity determination.

- Studying and comparing the results obtained.

1.27 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 2

LITERATURE REVIEW

Whole Plant

2.1 Ethnobotanical value of dry, fallen ovaries of *Bombax ceiba* L.

This study was performed to investigate the Ethnobotanical value of dry, fallen ovaries of *Bombax ceiba* L. and the relationship between the plant and human benefits. Author has found many different uses of various parts of investigated plant. Authors have found many uses of ovaries like in traditional Indian spicy rice preparation, cattle food, hypotensive agent, and antioxidant. Used in biriyani and as commercial animal food is new finding in this research. (Gopakumar, S. & R.Y. Bai, 2012).

2.2 Comparative Study on Physical and Mechanical Properties of Plywood Produced from Eucalyptus (Eucalyptus camaldulensis Dehn.) and Simul (*Bombax ceiba* L.) Veneers

Plywood becomes very important material for various structural purposes in Bangladesh and used as a substitute of solid wood. Therefore, the objective of this study was to determine and compare the physical and mechanical properties of plywood produced with veneers of eucalyptus and simul tree. The commercial urea formaldehyde resin was used for fabricating the panels. Physical properties i.e., density, moisture content, water absorption and thickness swelling; and mechanical properties i.e., modulus of elasticity (MOE) and modulus of rupture (MOR) of the panels were determined according to the procedure of ASTM standards. It was found that the density of eucalyptus and simul plywood was 879 and 536 kg/m respectively. Further, it was also observed that MOE and MOR of eucalyptus plywood were almost 2 and 2.5times higher respectively than those of simul plywood. These differences were attributed to the variation in properties of veneer wood species and the effect of veneer wood species on some physical and mechanical properties of plywood was found statistically different. (Nazmul, Nazrul, khandakar, 2012).

2.3 Possible modulation of FAS and PTP-1B signaling in ameliorative potential of *Bombax ceiba* against high fat diet induced obesity

Bombax ceiba Linn, commonly called as Semal, is used in various gastro-intestinal disturbances. It contains Lupeol which inhibits PTP-1B, adipogenesis, TG synthesis and accumulation of lipids in adipocytes and adipokines whereas the flavonoids isolated from *B. ceiba* has FAS inhibitory activity. The present study was aimed to investigate ameliorative potential of *Bombax ceiba* to experimental obesity in Westar rats, and its possible mechanism of action. (Gupta et al., 2013)

2.4 Biological Activity Study on a Malvaceae Plant: *Bombax ceiba*

The work described in this paper details the biological investigation on *Bombax ceiba*, species of *Malvaceae*. The methanol crude extract of *Bombax ceiba* was fractionated with kupchan method and *n*-hexane, carbon tetrachloride, chloroform fraction were made for screening the antimicrobial and antitumor potentials using disc diffusion method and brine shrimp lethality bioassay respectively. An established antibiotic (kanamycin, 30µg/disc) and cytotoxic agent (vincristine sulphate) were used to compare the results. From the graphs the concentration of methanolic crude extract give LC_{50} (50% mortality) value of 3.90mg/ml. LC_{90} was also determined from the graph to establish the therapeutic index and the value was found 150.0mg/ml. The four fractions were assayed for antimicrobial screening and the carbon tetrachloride fraction showed most prominent zone of inhibition against a number of bacterial and fungal strains. (Islam et al, 2011).

Root

2.5 Pharmacognostical and phytochemical studies on roots of *Bombax ceiba* Linn.

This study was investigated the Pharmacognostical, physicochemical and phytochemical study of the roots of *Bombax ceiba*. Pharmacognostical study included the macroscopic characters like size, color, surface characteristics, texture, fracture characteristics and odor of the roots. The intact root as well as powdered drug was studied under a microscope to analyze the cellular characteristics of the drug. Physicochemical parameter like extractive values, LOD, total ash, water soluble and acid insoluble ash, foaming index and hemolytic index of *Bombax ceiba* root powder were determined as per WHO guidelines. Preliminary phytochemical screening and qualitative chemical examination studies have been carried out using cyclohexane: diethyl ether: ethyl acetate as mobile phase. Chemical evaluation and TLC studies have shown presence of alkaloids, glycosides, flavonoids, steroids, saponins and tannins. The microscopic characters have shown presence of cork, cambium, xylem vessel, stone cells, starch grains, calcium oxalate crystals and phloem fibers. Microscopy analysis of the powder included the cork cells, fibers, calcium oxalate crystals and vessel. The presence of steroids was confirmed in HPTLC fingerprinting studies. (Chaudhary, Deore, 2014).

2.6 Aphrodisiac activity of *Bombax ceiba* Linn root extract

In this study, the aphrodisiac activity of *Bombax ceiba* Linn (Bombacaceae) root extract was investigated. The extract (400 mg/kg body wt./day) was administered orally by gavages for 28 days. Mount latency (ML), Intromission latency (IL), Ejaculation latency (EL), Mounting frequency (MF), Intromission frequency (IF), Ejaculation frequency (EF) and Post-Ejaculatory interval (PEI) were the parameters observed before and during the sexual behavior study at day 0, 7, 14, 21, and 28. The extract reduced significantly ML, IL, EL and PEI ($p < 0.05$). The extract also increased significantly MF, IF and EF ($p < 0.05$). These effects were observed in sexually active and inactive male mice. (Khadabadi 2014)

2.7 Anabolic Effect of *Bombax ceiba* L Root in Idiopathic Involuntary Weight - A Case Study

Bombax ceiba L is a popular plant among native communities for its medicinal properties. The root is specially used for debility and impotence. Here a case study of a patient has been reported of involuntary weight loss without any detectable cause who was administered 1.5 g of *B. ceiba* root powder with milk for 24 weeks. He regained his weight and achieved normal body mass index (19.9 Kg/m2) with 147% rise in fibrinolytic activity and marked improvement in his total antioxidant status without any undesirable side effects with its administration or withdrawal symptoms after its discontinuation. This case study, first time scientifically documents anabolic potential of *B. ceiba* root powder, which the indigenous communities have been utilizing since ages. (Verma *et al*, 2011).

2.8 Effect of *Bombax ceiba* L on spermatogenesis, sexual behavior & erectile function in male rats

A number of herbal drugs are advocated in the traditional Ayurvedic literature for the improvement of overall sexual function. Young roots of *Bombax ceiba* Linn. (Fam. Bombacaceae) also known as Semal Musli are used traditionally in Indian subcontinent as sexual stimulant. Its juice is considered nutritive and restorative tonic. Lyophilized aqueous extract of roots was studied for effect on sexual behavior and spermatogenesis in male albino rats. Administration of 100 mg Kg^{-1} body weight of aqueous extract influenced the five parameters evaluated *In Vivo. S*exual behavior analysis in the presence of a female rate, serum testosterone level, anabolic effects, epididymal sperm count and seminal fructose level were the parameters evaluated. In *B. ceiba* extract-treated animals, a gain in body and sexual organ weights was observed. Mount, intromission and ejaculation frequencies were significantly improved ($P < 0.05$). An increase in serum testosterone levels was also observed, but it was not statistically significant ($P > 0.05$). Seminal fructose content and epididymal sperm count were significantly improved as well. Penile erection index was also higher compared to control group animals. Hesitation time was significantly reduced ($P < 0.01$), and copulatory rate was doubled in treated animals compared with control group animals. (Yadav et al, 2012)

Stem Bark

2.9 Antiangiogenic activity of lupeol from *Bombax ceiba*

This study was investigated the antiangiogenic activity of lupeol from *Bombax ceiba*. In the search for antiangiogenic agents from medicinal plants used in Vietnam, a methanol extract of the stem barks of *Bombax 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 afforded lupeol as an active principle. At 50 and 30 µg/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. (Kim *et al*., 2003)

2.10 *In Vivo* Antioxidant and Immunomodulatory Activity of *Bombax ceiba* bark - Focusing on its Invigorating Effects

The following study evaluated the antioxidant and Immunomodulatory activity of methanol extract of the bark of *Bombax ceiba* in normal and immune suppressed mice models. *In Vivo* Immunomodulatory and antioxidant activity of *Bombax ceiba* methanol extract was evaluated by assessing its effect on Hemagglutinin antibody (HA) titer, delayed type of hypersensitivity (DTH) response, hematological profile (Hb,WBC, RBC), lipid per oxidation (LPO), reduced glutathione (GSH),superoxide dismutase (SOD), catalase (CAT) and cytokine release. The animals treated with *Bombax ceiba* methanol extract showed increase in antibody titer values 11.2 ± 0.30 and 13.1 ± 0.27at 150 and 300 mg/kg (p.o.) dose and DTH reaction induced by SRBC was also found significant ($P < 0.001$). Also it caused increase in hematological profile, GSH, SOD, CAT activity and significantly decreased LPO levels in cyclophosphamide-induced immunosuppressed mice. The extract treated animals showed a significant up regulation of (IL-6 and TNF-α) cytokines in comparison to control group animals. These findings suggested that the methanol extract of *Bombax ceiba* possessed promising immunostimulant properties which could be ascribed, in part, to its anti-oxidant capacity. (Wahab et al., 2013)

2.11 Potential anti-diabetic activity of *Bombax ceiba*

In this study *Bombax ceiba* bark extract was evaluated for its hypoglycemic and hypolipidemic potential through normal and streptozotocin-induced diabetic rats administered with graded oral doses (200, 400, 600 mg/kg/day) for 21 days. The results showed that a dose of 600 mg/kg of *B. ceiba* extract is the most effective to cause significant ($p<0.001$) hypoglycemic and/or hypolipidemic effects on streptozotocin-induced diabetic rats. This dose also significantly ($p<0.001$) lowered the total cholesterol and triglyceride level in severely diabetic rats. Phytochemical and GC-MS studies confirmed the presence of the triterpinoids 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 and Talele, 2013)

2.12 Screening of Antibacterial Activity of Aqueous Bark Extract of *Bombax ceiba* Against Some Gram Positive & Gram Negative Bacteria

The objective of this study is to evaluate the antibacterial activity of aqueous extracts of the bark of *Bombax ceiba.* To evaluated for their antibacterial activity using the Pour plate method. It was tested against six medically important bacterial strains, namely Gram-positive Bacteria (*Bacillus subtilis, Bacillus aureus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, K *pneumoniae*, *and Pseudomonas aeruginosa)*. The potency of the microorganisms to the aqueous extracts of *Bombax ceiba* was compared with standards drug i.e. Gentamicin. The aqueous extract was more significant against Gram-positive bacteria than against Gram-negative bacteria. The 100 µg/ml showed the best antibacterial activity as compared to the standard. The aqueous extract of *Bombax ceiba* which contains Tannin has shown significant antibacterial activity due to the presence of tannins. (Kuthar et al., 2015)

2.13 Hypotensive activity and toxicology of constituents from *Bombax ceiba* stem bark

A novel constituent, Shamimicin, 1, 1’ -bis-2-(3,4-dihydroxyphenyl)-3,4-dihydro-3,7-dihydroxy-5-O-xylopyranosyloxy-2H-1-benzopyran along with lupeol, which possesses potent hypotensive activity, has been isolated from *Bombax ceiba* stem bark. BCBMM--one of the most active

hypotensive fractions has revealed its adverse effects on heart, liver and kidneys of mice at the dose of 1000 mg/kg/d. (Zikr-ur-Rehman et al., 2003)

Flower:

2.14 Cardioprotective effect of *Bombax ceiba* flowers against acute Adriamycin induced myocardial infarction in rats

This study was designed to evaluate the cardioprotective potential of aqueous flower extract of *Bombax ceiba* L., Malvaceae (BC), on the basis of biochemical and histopathological parameters in Adriamycin induced myocardial infarction in rats and to compare with vitamin E, a known cardioprotective antioxidant. Male Wister rats were used as *In Vivo* model for the study. BC was administered orally to Wister rats at different doses (150 mg/kg, 300 mg/kg and 450 mg/kg) for six days/week for four weeks. Thereafter, all the groups except saline were administered Adriamycin (20 mg/kg). There was a significant decrease in myocardial superoxide dismutase, catalase and reduced glutathione in animals treated with Adriamycin. Concurrently marked increase in extent of lipid peroxidation was reported. Co-treatment of BC/vitamin E and Adriamycin resulted in an increase in the cardiac antioxidant enzymes and reduction in lipid peroxidation as compared to Adriamycin -treated animals. Adriamycin showed significant decrease ($p<0.001$) in the level of cardiac marker enzymes [Lactate dehydrogenase (LDH) and Serum glutamic oxaloacetic transaminase (SGOT)] in heart homogenate with corresponding increase in their level in serum. In BC/vitamin E treated groups significant increase ($p<0.001$) of LDH in heart homogenate and decrease of SGOT and LDH in serum were observed. Microscopic studies in Adriamycin -treated animals revealed mitochondrial swelling, leukocyte infiltration, lipid inclusions and myofibrillar loss whereas the pre-treatment with BC/vitamin E led to a lesser degree of Adriamycin -induced histological alterations. These findings suggest that aqueous flower extract of BC has protective effect against Adriamycin -induced cardiotoxicity and may have potential as a cardioprotective agent. (Revista Brasileira de, 2011)

2.15 Hepatoprotective Activity of *Bombax ceiba* Linn against Isoniazid and Rifampicin-induced Toxicity in Experimental Rats

In this study the Hepatoprotective activity of methanolic extract of flowers of *Bombax ceiba* L. (MEBC) was investigated against hepatotoxicity produced by administering a combination of two anti-tubercular drugs Isoniazid and Rifampicin for 10 and 21 days by intra peritoneal route in rats. MEBC were administered at three graded dose i.e. 150, 300 and 450 mg/kg through intra peritoneal route 45 min prior to anti-tubercular challenge for 10 and 21 days. MEBC was evident in the all doses as there was a significant decrease in AST, ALT, ALP, and Total Bilirubin levels, but increased the level of total protein in comparison to control. MEBC significantly decreased the level of TBARS and elevated the level of GSH at all doses as compared to control. Histology of the liver section of the animals treated with MEBC improved the hepatotoxicity caused by anti-tubercular drugs. The results obtained from the analysis of biochemical parameters and histopathological studies, enabled to conclude that the MEBC were not able to revert completely the hepatic injury induced by INH + RIF, but it could limit the effect of INH + RIF to the extent of necrosis. (Ravi et al, 2010)

Fruits

2.16 Curative treatment with extracts of *Bombax ceiba* fruit reduces risk of calcium oxalate urolithiasis in rats

Drawbacks of presently available treatments for urolithiasis necessitate finding the treatment of hyperoxaluria specifically aimed at reduction in oxalate excretion. Interestingly, many Indian tribes use *Bombax ceiba* L. (Bombacaceae) fruits as a traditional medicine for the treatment of urinary stones. The present study investigated the efficacy of *B. ceiba* fruit extracts as curative agents in experimentally induced calcium oxalate urolithiatic rats. Calcium oxalate lithiasis was induced in rats by oral administration of 0.75% ethylene glycol for 14 consecutive days. Treatments with aqueous and ethanol extract of *B. ceiba* fruit (400 mg/kg body weight) was

performed in the same manner for further 14 consecutive days. Cystone (750 mg/kg body weight) was used as reference anti-urolithiatic drug. The urinary excretion and kidney deposition of offending salt components, and serum biochemical parameters were investigated. Oral administration of ethylene glycol resulted in hyperoxaluria and increased renal excretion of calcium and phosphate. However, supplementation with aqueous and ethanol extracts of *B. ceiba* fruit significantly ($p < 0.05$) reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in kidneys of calculogenic rats was also significantly lowered with curative treatment of aqueous and ethanol extract. The results indicate that the fruit of *B. ceiba* is endowed with lithontriptic activity warranting further development for curative treatment of urolithiasis. (Gadge and Jalalpur, 2012)

2.17 Diuretic Effects of Young Fruit Extracts of *Bombax ceiba* L. in Rats

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), furosemide 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 furosemide and hydrochlorothiazide. 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 and Gadge, 2011)

2.18 A study on proximate analysis and antimicrobial properties of *Bombax ceiba* pentandra fruit and spike extracts

This study investigated the antibacterial study of five solvent extracts of spike and young fruits using aqueous, methanol, ethyl acetate, chloroform, and Hexane against five bacterial species namely *Escherichia coli, Bacillus Subtilis, Staphyolococcus aureus, Pseudomonasaerogenosa* and *Shigella flexnerri.* Also Antimycotic study was performed using the five solvent extracts against the fungal cultures namely *Candida albicans, Fusarium oxysporum, Cladosporium bantia, Alternariabrassicae, Curvalaria lunata*. Among all the solvent extracts, methanol extract of fruit and spike exhibited significant Antibacterial and Antimycotic activity. Presence of higher concentration of Polyphenolics and flavonoids compounds in the methanol extract may be responsible for the effective antimicrobial property. (Nagamani, Avinash, 2015)

Leaf

2.19 Development of the HPTLC Densitometric Method for Estimation of Quercetin in *Bombax ceiba* L Leaves

Bombax ceiba L. is a medicinally valuable herb in the Ayurvedic and traditional systems of medicine. Various activities have been reported in almost all parts of *Bombax ceiba*, some of these include hypertensive, antioxidant, hypoglycemic, hepatoprotective, and antipyretic. Quercetin, one of the most important flavonoids is active against various cardio vascular diseases, cancer, tuberculosis, neurological diseases, cataract etc. In the present study High Performance Thin Layer Chromatography method has been developed for detection and quantification of Quercetin in *Bombax ceiba* leaves. Increasing serial dilutions of reference standard Quercetin (200 to 1000 µg/ml) were scanned at 366 nm to detect and quantify the concentrations of Quercetin in the test sample. The estimated value obtained from the same was 5.38% Quercetin in powdered leaf sample. The method provided a rapid and easy approach for detection and the quantization of the bio-marker Quercetin. (Gupta et al, 2014)

2.20 Hypotensive, hypoglycemic and toxicological studies on the flavonol C-glycoside shamimin from *Bombax ceiba*

Shamimin, a C-flavonol glucoside from *Bombax ceiba* leaves showed significant potency as a hypotensive agent at the doses of 15 mg/kg, 3 mg/kg, 1 mg/kg and significant hypoglycemic activity at 500 mg/kg in Sprague-Dawley rats. Further studies revealed that it did not cause any mortality in mice at the dose of 1 g/kg but in rats 500 mg/kg is a lethal dose. Aqueous and methanolic extracts of *Bombax ceiba* leaves and one of its fractions were also subjected to pharmacological and toxicological screening. (Saleem et al., 1999)

2.21 An Investigation on Preliminary Phytochemical and Safety Profiles of Methanolic Leaf Extract of *Bombax ceiba*

The present study was aimed to evaluate phytochemical constituents and the safety of methanolic leaf extract of *Bombax ceiba* (MEBC) by determining their potential toxicity after acute and 28-day repeated dose administration in Westar Albino rats. The phytochemical analysis was done by standard laboratory grade reagents. Acute and 28-day repeated dose oral toxicity studies were performed by the following Organization for Economic Co-operation and Development (OECD) test guidelines 423 and 407 respectively. The phytochemical screening revealed the presence of steroid, alkaloid, tannin, phenols, flavonoids, in methanol soluble fractions. Fats and fixed oils were undetected in the extracts. In acute toxicity study no treatment related death or toxic signs were observed with MEBC administration. In repeated dose study no significant differences in body weight changes and hematology was observed between control and MEBC groups. Conversely there was a decrease in serum glucose and cholesterol levels and an increase in protein levels in treated rats compared to control. No gross pathological findings and difference in relative organ weights were observed between control and treated rats. Histopathological examination revealed no abnormalities with the test drug treatment. In conclusion *Bombax ceiba* was found to be non toxic in tested doses and experimental conditions. (Chandiran et al, 2015)

2.22 Bioassay-guided studies on *Bombax ceiba* leaf extract: isolation of shamimoside, a new antioxidant xanthone *C*-glucoside

Bioassay-guided isolation studies on the methanolic extract of the leaves of *Bombax ceiba* employing DPPH antioxidant assay led to the isolation of a new xanthone *C*-glucoside, shamimoside (**2**), along with three known constituents, mangiferin (**1**), stigma-5-en-3-*O*-β-glucoside, and β-amyrins. The structure of shamimoside has been elucidated through extensive spectroscopic methods, including 1D and 2D NMR experiments, as 4-*C*-β-D-glucopyranosyl-1,3,6,8-tetrahydroxy-7-*O*-(p-hydroxybenzoyl)-9H-xanthen-9-one. It is the first naturally occurring xanthone containing a benzoate moiety directly attached to an aromatic ring. Polar extracts and fractions demonstrated better antioxidant activity, and mangiferin was found to be more potent than shamimoside in this assay. (Versiani et al., 2012)

Chapter 3

MATERIALS & METHODS

3.1. Preparation of the Plant Extract for the Experiments

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 ^{0}C) for 2 hours to make suitable for grinding purpose.

3.1.3 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed air tight glass containers till extraction with necessary markings for identification and kept in cool, dark and dry place for the investigation. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 900 gm which was measured using electronic balance and it was found to be 900 gm.

3.1.4 Extraction procedure

The powdered plant materials were submerged into 1000 ml Dichloromethane in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. This process is termed as maceration. The major portion of the extractable compounds of the plant materials were dissolved in the solvent.

3.1.5 Filtration of the Extract

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by

using cotton and filter paper. Then the filtrate, was taken into a volumetric flask and covered with aluminum foil paper, was prepared for rotary evaporation.

3.1.6 Solvent Evaporation

The filtrate was kept in rotary evaporator for complete evaporation of the solvent. The solution was also kept in the hot plate and stirred frequently for solvent evaporation. After running this procedure, a gummy and oily extraction was obtained which was preserved in proper manners.

3.2 Principle of a Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure").Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Büchi in 1957. Other common evaporator brands are Heidolph, LabTech, Stuart, Hydrion Scientific, SENCO, IKA and EYELA. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L-50L) versions are used in pilot plants in commercial chemical operations.

Figure 3.1: Drying of extract using rotary evaporator

3.3. *In vitro* Antibacterial Screening

3.3.1. Preamble

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

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, and 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. Bacterial inoculums are applied to the surface of a large agar plate. Antibiotic discs and disc of test materials are placed on the inoculated agar surface. Plates are incubated for 16–24hr at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The zones of growth inhibition are measured to the nearest millimeter around each of the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. However, no distinction between bacteriostatic and bactericidal activity can be made by this method. (Barry, 1976)

3.3.2. Principle of Disc Diffusion Method

The Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient.

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.

This test must be rigorously standardized since zone size is also dependent on inoculum size, medium composition, temperature of incubation, excess moisture and thickness of the agar. If these conditions are uniform, reproducible tests can be obtained and zone diameter is only a function of the susceptibility of the test organism.

Zone diameter can be correlated with susceptibility as measured by the dilution method. Further correlations using zone diameter allow the designation of an organism as “susceptible”, “intermediate” or “resistant” to concentrations of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy.

In the present study the crude Dichloromethanolic extract of *Bombax ceiba* was tested for antibacterial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required. (Bauer et al., 1966)

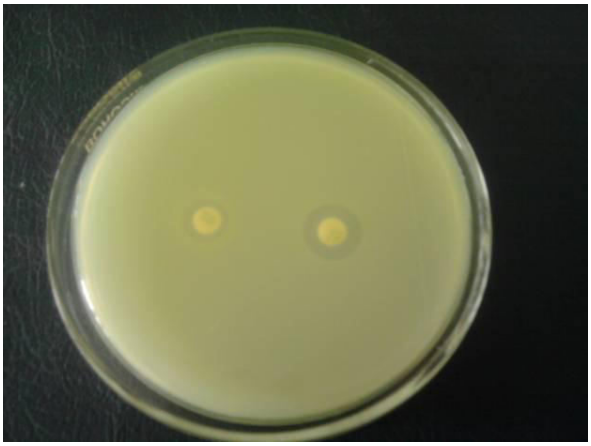


Figure 3.2: Disc Diffusion Method

3.3.3. Experimental

3.3.3.1. Apparatus and Reagents

- Filter paper discs
- Petri dishes
- Inoculating loop
- Sterile cotton
- Sterile forceps
- Spirit burner
- Micropipette & micropipette tips
- Reagent bottles
- Screw cap test tubes
- Eppendorf tube & vial
- Spreader & Spatula
- Nose mask and Hand gloves
- Laminar air flow hood
- Autoclave
- Incubator
- Hot Air Oven
- Electronic Balance
- Refrigerator
- Nutrient Agar Medium
- Ethanol
- Chloroform

3.3.3.2. Test materials

3.3.3.2.1. Test samples

Crude Dichloromethanolic extract of *Bombax ceiba*

3.3.3.2.2. 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. Gram positive bacteria and fungi were taken for the test and they are listed in the Table 3.1

Table 3.1 List of Test Organisms

1. ***Shigella dysenteriae***
2. ***Pseudomonus aureas***
3. ***Saccharromyces cerevisiae***

Shigella dysenteriae

- *Shigella dysenteriae* is a species of the rod shaped bacterial genus Shigella.
- Shigella can cause shigellosis (bacillary dysentery)
- Shigella is Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.
- *Shigella dysenteriae,* spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents.

Pseudomonas aureus

- *Pseudomonas aureus* is a common Gram-negative bacterium that can cause disease in plants and animals, including humans.
- It is a Gram-negative, aerobic, bacillus bacterium with unipolar motility.

- *Pseudomonas aureus* is considered by many as a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion.
- *Pseudomonas aureus* uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis.
- *Pseudomonas aureus* typically infects the airway, urinary tract, burns, wounds, and also causes other blood infections.

Saccharromyces cerevisiae

- *Saccharomyces cerevisiae* is a eukaryotic microbe. More specifically, it is globular-shaped, yellow-green yeast belonging to the Fungi kingdom.
- Natural strains of the yeast have been found on the surfaces of plants, the GI tracts and body surfaces of insects and warm-blooded animals, soils from all regions of the world and even in aquatic environments. Most often it is found in areas where fermentation can occur, such as the on the surface of fruit, storage cellars and on the equipment used during the fermentation process.

3.3.4. Culture medium and their composition

Table 3.2: Different Culture Medium & Components

Nutrient Agar Medium		Nutrient Broth Medium	
Ingredients	**Amounts**	**Ingredients**	**Amounts**
Bacto Peptone	0.5 gm	Bacto beef extract	0.3 gm
Sodium Chloride	0.5 gm	Bacto peptone	0.5 gm
Bacto Yeast Extracts	1.0 gm	Dist. Water q.s to	100 ml
Bacto Agar	2.0 gm	P^H	7.2 ± 0.1 at 25^0C
Distilled Water q.s. to	100 ml		
P^H	7.2 ± 0.1 at 25^0C		

Muller – Hunton Medium		Tryptic Soya Broth Medium (TSB)	
Ingredients	**Amounts**	**Ingredients**	**Amounts**
Beef infusion	30 gm	Bacto tryptone	1.7 gm
Casamino acid	1.75 gm	Bacto soytone	0.3 gm
Starch	0.15 gm	Bacto dextrose	0.25 gm
Bacto agar	1.70 gm	Sodium chloride	0.5 gm
Distilled water q.s. to	100 ml	Dipotassium hydrogen Phosphate	0.25 gm
P^H	7.3 ± 0.2 at 25^0 C	Distilled water q.s. to	100 ml
		P^H	7.3 ± 0.2 at 250 C

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

3.3.4.1. Sterilization of Petri Dishes

Petri dishes having 130 mm diameter were used in this test. The Petri dishes were placed in the hot air oven at 150ºc temperature for 15 minutes for sterilization. After sterilization, the Petri dishes were transferred inside the laminar air flow cabinet to avoid contamination.

3.3.4.2 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.

Figure 3.3: Nutrient Agar in Culture Bottle

3.3.5. 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 glass wares 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.

3.3.6. 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.

3.3.7. 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.

3.3.8 Stock Solution Preparation

To prepare the stock solution of samples of 300 µg /disc and 600 µg/disc concentrations, 0.3 gm and 0.6 gm samples were dissolved in 10 ml Dichloromethane in two different test tubes respectively. Then the solutions in the test tubes were shaken to dissolve the sample properly.

3.3.9 Preparation of the Isotonic Solution

A 0.9% isotonic solution had to be prepared. This was prepared by weighing 0.9 g of Sodium chloride (NaCl) and by dissolving the measured Sodium chloride in 100 ml of distilled water. The isotonic solution was also autoclaved at 121°c for 15 minutes.

3.3.10 Dilution of the Test Micro-organisms

Previously cultured Petri dishes of the test microorganisms were assembled. At first, an inoculating loop was sterilized in a Bunsen burner. Then it was used to scrape a small colony of a specific species of microorganism from its culture. Now, the microorganism on the loop was transferred to a sterilized eppendorf tube, already containing 1 ml of isotonic solution. Then, the inoculating loop was resterilized and used to transfer another species of microorganism to a fresh eppendorf tube already filled with isotonic solution. In this way all the microorganisms were transferred to fresh eppendorf tubes and thus were made ready for the test. The eppendorf tubes were then applied on a vortex mixer for proper mixing of microorganism with the isotonic solution.

3.3.11. Preparation of Discs

3.3.11.1. 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, Kanamycin (30µg/disc) standard disc was used as the reference.

3.3.11.2. 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.

3.3.11.3. 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 Petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

3.3.11.3.1. Preparation of sample discs

Dichloromethanolic extracts of *Bombax ceiba* were tested for antimicrobial activity against two gram positive bacteria and a Fungi species the amount of sample per disc was 300 µg and 600 µg.

3.3.12. 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.

3.3.12.1. 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 ^{0}C 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^{0}C for 24 hours.

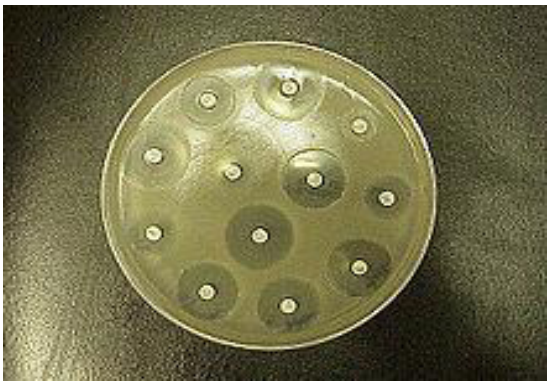


Figure 3.4: Filter paper discs

3.3.13 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.

3.4. *In vitro* Determination of the Antioxidant Activities

3.4.1. Preamble

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 (0_2.) anion, hydrogen peroxide (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.

3.4.2 Principle

Aluminum chloride colorimetric method was used for flavonoids determination. 1.5 ml extract (1mg/ml) in Dichloromethane was taken. After that, 6 ml distilled water was added with 0.45 ml Sodium Nitrate (5%w/v). The mixture was incubated for 6 minutes, then 0.45 ml Aluminium 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. The calibration curve was prepared by preparing Quercetin solutions at various concentrations in Dichloromethane. The concentration of flavonoids was expressed in terms of mg/100 g of sample. (Ainsworth and Gillespie, 2007)

3.4.3 Reagents

- 10% Aluminum Chloride ($AlCl_3$) solution
- Sodium Nitrate (5%w/v) and Sodium hydroxide (4%)
- Dichloromethane
- Quercetin (Analytical or Reagent grade)

3.4.4 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.

3.4.5 Preparation of 1M Potassium acetate solution

9.815 gm of potassium was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.4.6. Preparation of Standard solution

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

Table: 3.3 Preparation of Standard Solution

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by Dichloromethane	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

3.4.7 Preparation of Extract solution

1.5 ml extract in Dichloromethane was taken. The concentration of the solution was 1mg/ml of plant extract.

3.4.8 Experimental procedure

- 1.5 ml of each plant extracts or standard of different concentration solutions were taken in different test tubes and 2 ml of Dichloromethane 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 Aluminium 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 against blank.
- The Total content of flavonoid compounds in plant Dichloromethane extract was Quercetin equivalent.

3.5 Brine Shrimp Lethality Bioassay

3.5.1. Preamble

Bioactive compounds are always toxic to living body at some higher doses and it justifies the statement that 'Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses'. Brine shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compound of the natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening and fractionation in the discovery of new bioactive natural products. This bioassay indicates Cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal & anti-tumor etc. of the compounds. Brine shrimp lethality bioassay technique stands superior to other Cytotoxicity testing procedures because it is rapid in process, inexpensive and requires no special equipment or aseptic technique. It utilizes a large number of organisms for statistical validation and a relatively small amount of sample. Furthermore, unlike other methods, it does not require animal serum.

3.5.2 Materials

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

Test samples that used in this experiment were Crude Dichloromethanolic extract of *Bombax ceiba.*

3.5.3. Principle

Brine shrimp eggs are hatched in simulated sea water to get nauplii. Test samples are prepared by dissolving in DMSO (Dimethyl Sulfoxide) and by the addition of calculated amount of DMSO (Dimethyl Sulfoxide), desired concentration of the test sample is prepared. The nauplii are counted by visual inspection and are taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations are added to the premarked vials through micropipette. The vials are then left for 16 hours and then the nauplii are counted again to find out the cytotoxicity of the test agents.

3.5.4. Procedure

3.5.4.1. Preparation of seawater

38 gm sea salt (pure NaCl) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

3.5.4.2. Hatching of brine shrimps

Artemia salina Leach (Brine Shrimp eggs) collected from pet shops was used as the test organism. Artificial seawater was taken in the small tank and Shrimp eggs were added to one side of the tank and then that side was covered. The tank was kept under constant aeration for 48 hrs to hatch the Shrimp and to be matured as nauplii. The hatched Shrimps were attracted to the lamp through the perforated dam and with the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of Brine solution.

3.5.4.3 Preparation of test solutions

2mg of each sample was dissolved in 60 µg of DMSO (Dimethyl Sulfoxide). A series of solutions of lower concentrations were prepared by serial dilution with DMSO (Dimethyl Sulfoxide). From each of these test solutions 30 µg were added to pre-marked glass vials/test tubes containing 5 ml of seawater and 10 Shrimp nauplii. So, the final concentration of samples

in the vials/test tubes were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.5 µg/ml, 3.125µg/ml and 1.5625µg/ml for 9 dilutions.

3.5.4.4 Preparation of Controls

Vincristine sulphate served as the positive control. 0.2 mg of vincristine sulphate was dissolved in DMSO (Dimethyl Sulfoxide) to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO (Dimethyl Sulfoxide) to get 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, 0.0390 µg/ml. The control groups containing 10 living Brine Shrimp nauplii in 5 ml simulated seawater received the positive control solutions.

As for negative control, 30 µg of DMSO (Dimethyl Sulfoxide) was added to each of the pre-marked test tubes containing 5 ml of simulated seawater and 10 Shrimp nauplii. The test was considered invalid if the negative control showed a rapid mortality rate and therefore has to conduct again. The test tubes (containing nauplii) were then maintained at room temperature for 24 hrs under the light for observing the survival rate.

3.5.4.5 Counting of nauplii and analysis of data

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period. However, LC_{90} values were also calculated in the similar way for all fractions and the reference cytotoxic drug vincristine sulphate.

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

Chapter 4

RESULTS & DISCUSSION

4.1 *In vitro* Antimicrobial Screening

The antimicrobial activities of dichloromethanolic extract of *Bombax ceiba* was examined in the present study. The results of antibacterial activity are given in Table 4.1 which clearly shows that the dichloromethanolic extract of *Bombax ceiba* possesses some antibacterial activity against the entire tested organisms.

Table 4.1: The Antibacterial activity (*in vitro*) of *Bombax ceiba* Dichloromethane extract & Standard Kanamycin discs

Serial No.	Name of Test Organism	Characteristics	Diameter of the zone of inhibition (mm)		
			Dichloromethane extract of *Bombax ceiba*		Standard (Kanamycin)
			300 µg/disc	600 µg/disc	30 µg/disc
01	*Shigella dysenteriae*	Gram Negative Bacteria	10 mm	7 mm	24 mm
02	*Pseudomonas aureus*	Gram Negative Bacteria	10 mm	7 mm	23 mm
03	*Saccharomyces cerevisiae*	Yeast	7 mm	8 mm	12 mm

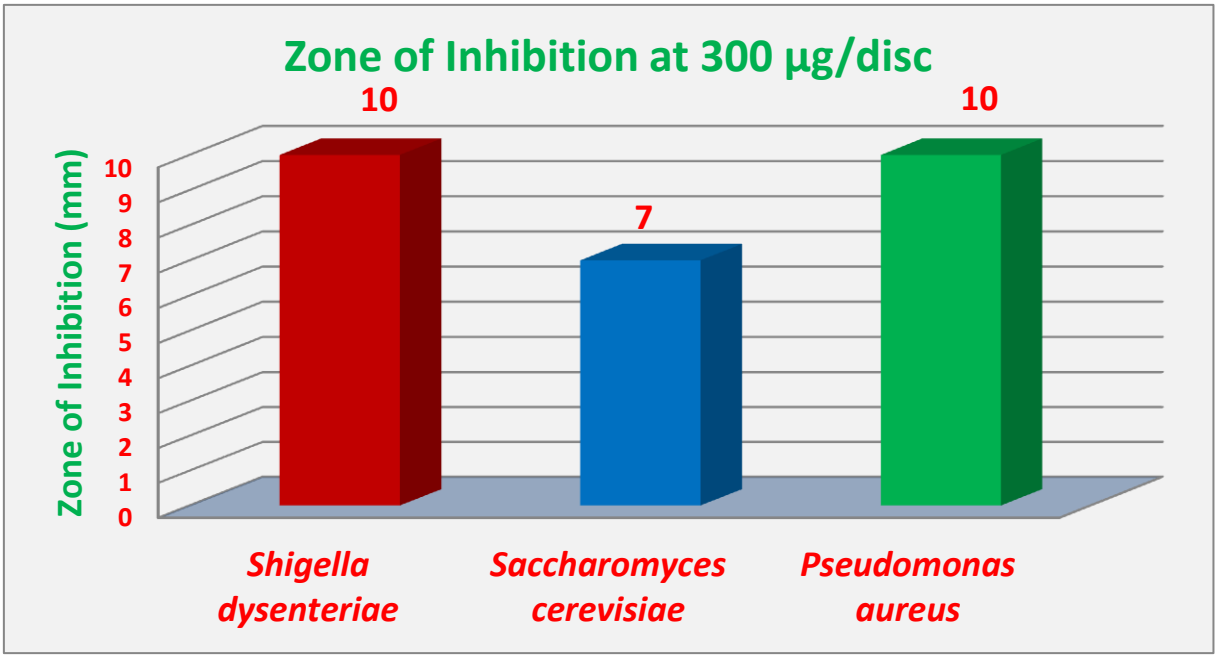


Figure 4.1: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms to Dichloromethane extract with concentration 300 µg/disc

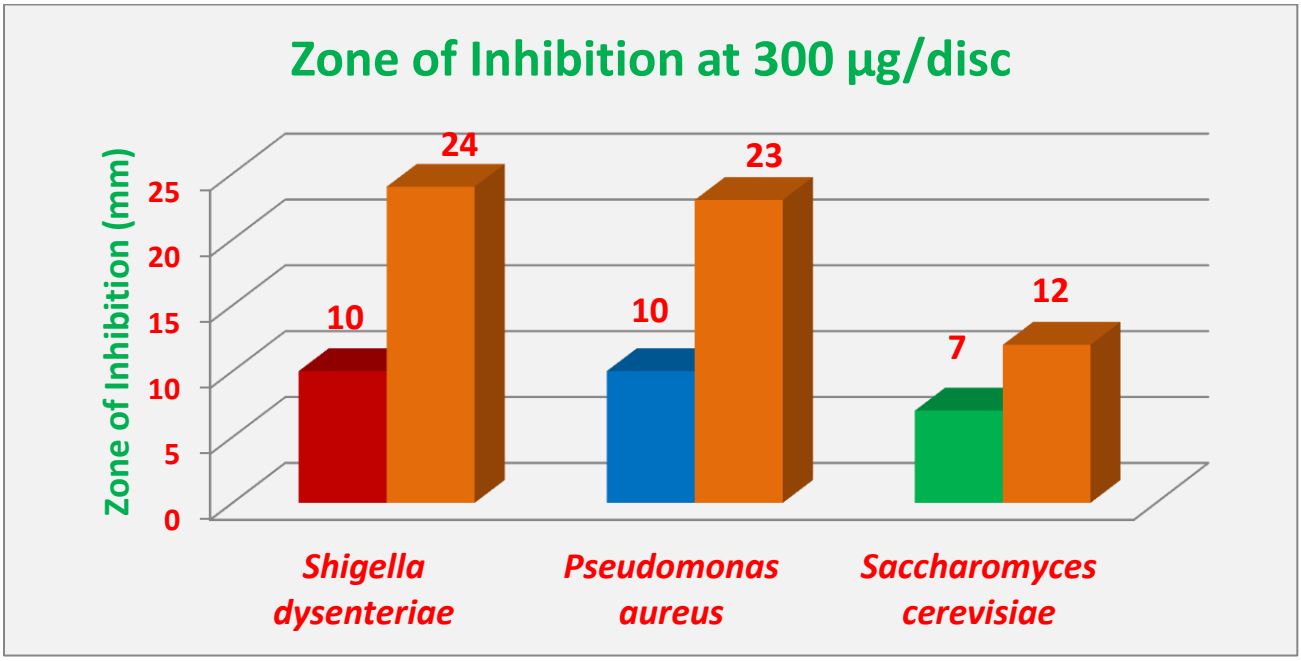


Figure 4.2: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms to Dichloromethane extract with concentration 300 µg/disc against Kanamycin standard

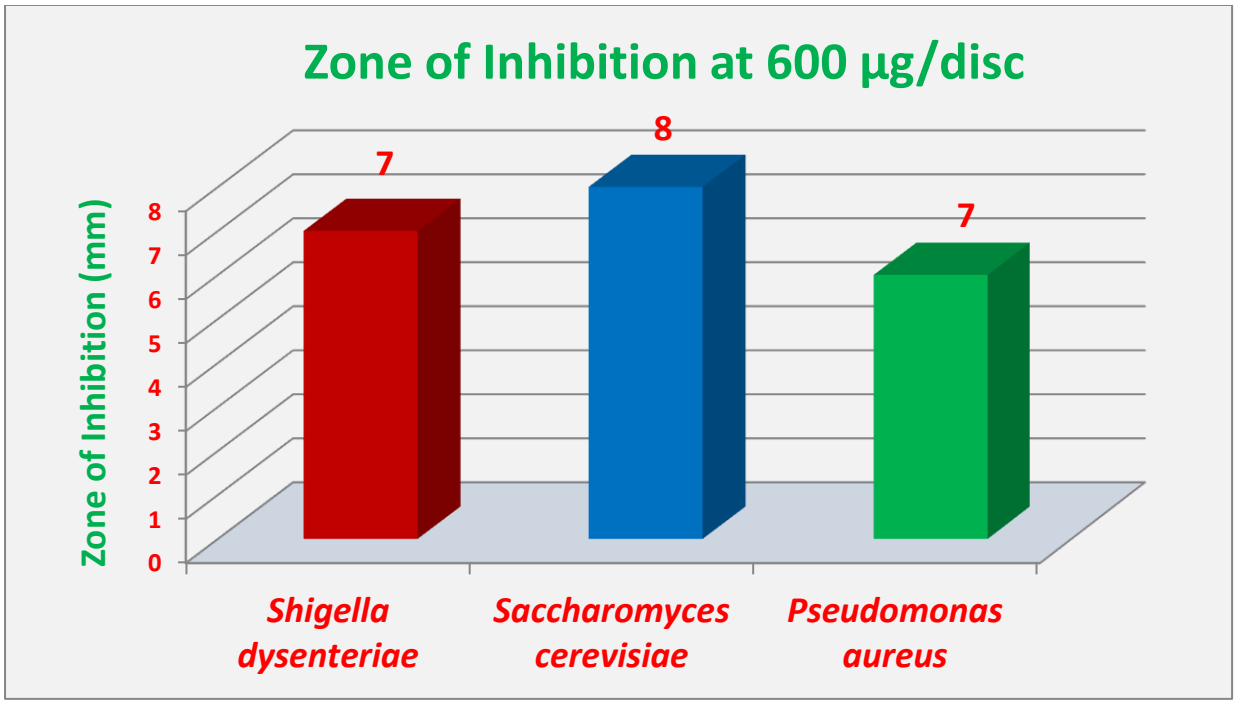


Figure 4.3: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms to Dichloromethane extract with concentration 600 µg/disc

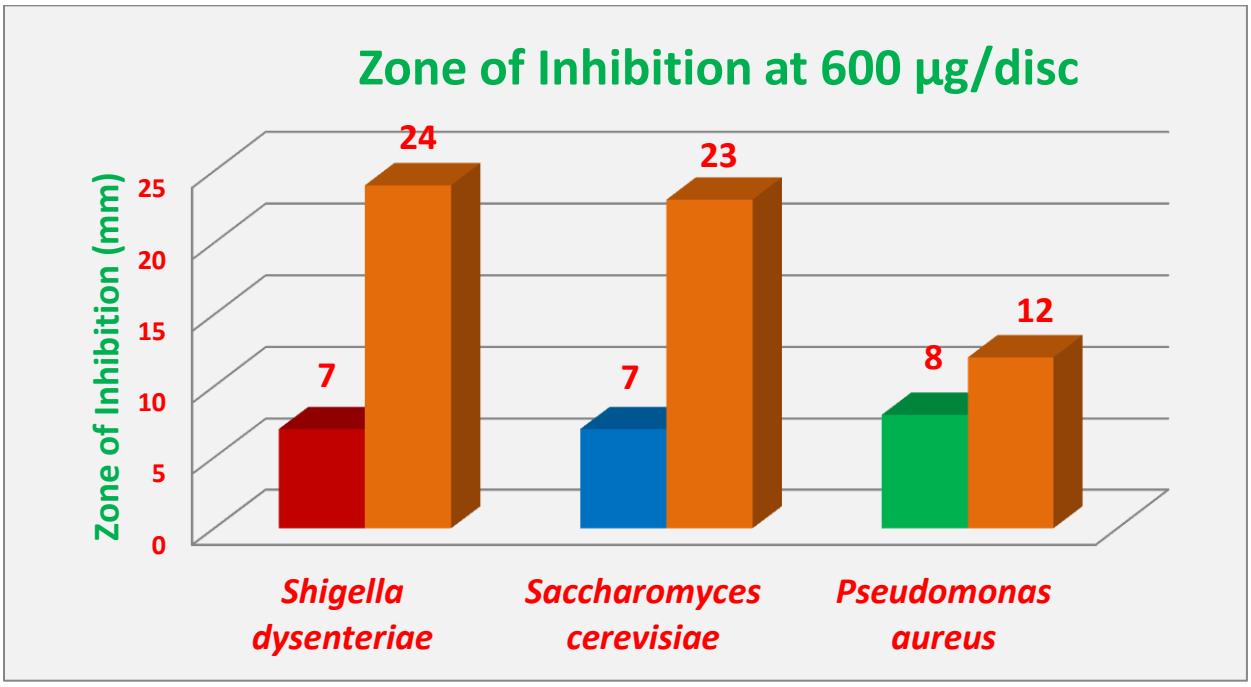


Figure 4.4: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms to Dichloromethane extract with concentration 600 µg/disc against Kanamycin standard

4.2 *In vitro* Determination of the Antioxidant Activities

The flavonoid content of dichloromethanolic extract of *Bombax ceiba* was examined in the present study. The results of this study are given in Table 4.2 which clearly shows that the dichloromethanolic extract of *Bombax ceiba* contains some flavonoid contents. The standard used here is Quercetin. From the calibration curve of Quercetin we get an equation that has been used to determine the flavonoid content in the sample of Dichloromethane extract of *Bombax ceiba*.

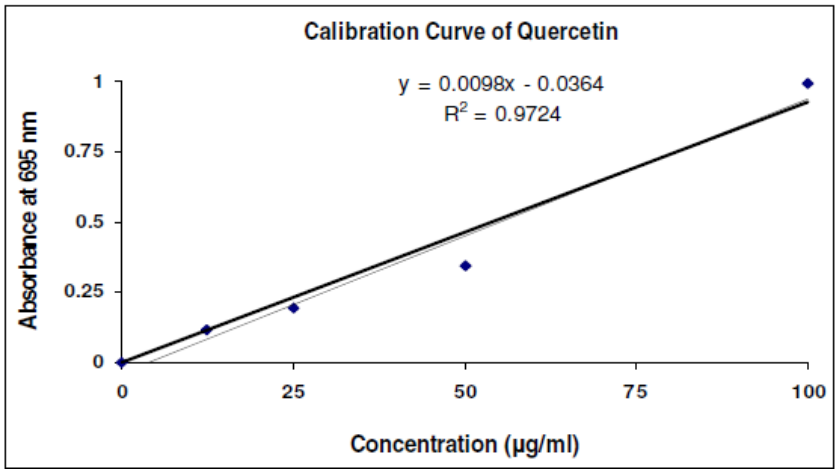


Figure 4.5: Calibration Curve of Quercetin at 695 nm

Table 4.2 Flavonoid content (mg/g) in Dichloromethane Extract of *Bombax ceiba* at 695 nm

Sample of Dichloromethane extract of *Bombax ceiba*	Absorbance (Y)	Equation from Standard Curve	Flavonoid Content (x) (mg/g, Quercetin equivalent)	Average ± SD
Reading1	0.045	**y=0.0098x -0.0364**	8.306	**7.847± 0.649**
Reading 2	0.036		7.388	

4.3 Brine Shrimp Lethality Bioassay

Every chemical that have effect on animal body will also have lethal effect. The lethality effect of dichloromethanolic extract of *Bombax ceiba* was examined in the present study. The results of this study are given in Table 4.3 which clearly shows that the dichloromethanolic extract of *Bombax ceiba* have lethal properties. The Brine Shrimp is used here as test animal model. From the collected data a curve of Brine Shrimp lethality bioassay has been produced. From the curve we get an equation that has been used to determine the LC_{50} (on which concentration 50% of test animal model is dead) for the sample of Dichloromethane extract of *Bombax ceiba*.

Table 4.3: Brine Shrimp Lethality Bioassay

Concentration, C (µg/ml)	Log C	%Mortality	LC_{50} (µg/ml) ± SD
400	2.60206	**80**	**19.85 ± 0.89418**
200	2.30103	**80**	
100	2	**70**	
50	1.69897	**60**	
25	1.39794	**50**	
12.5	1.09691	**40**	
6.25	0.79588	**40**	
3.125	0.49485	**30**	
1.5625	0.19382	**20**	
0	0	**20**	

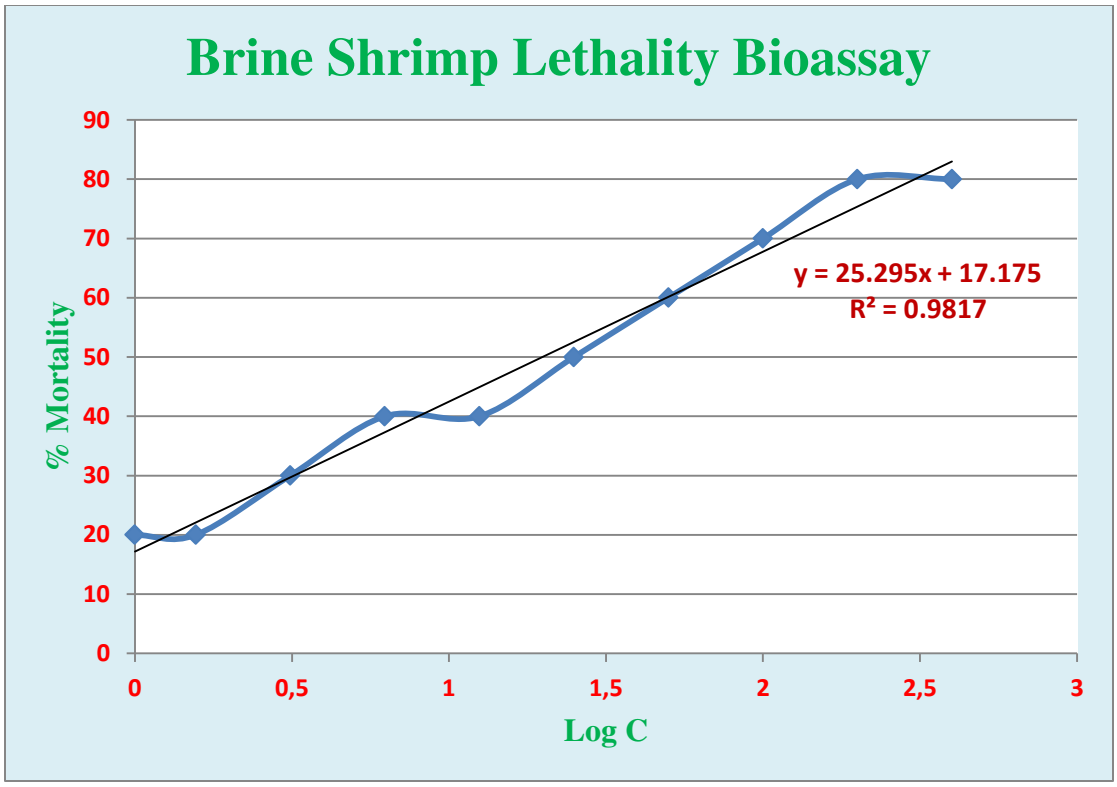


Figure 4.6: Curve of Brine Shrimp Lethality Bioassay

4.4 Discussion

Nature has been kind enough to humans by providing a wide range of plants having therapeutic potential. Screening of plants for isolation and identification of the natural bioactive products not only enrich the therapeutic compendium but also provide a cheaper, effective and safe alternative approach for treating diseases. It is a combined effort of botanists and clinicians for utilizing these plants for research and developing new drugs in controlling the growing epidemic or dreadful diseases such as myocardial infarction, diabetes, cancer, stroke etc.

The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plants extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plants are commonly having antimicrobial properties. The presence of saponins supports the fact that plant has cytotoxic effects such as intestinal permealization.

Bombax ceiba is a medicinal plant enriched with various chemical constituents having different medicinal activities. Gum from the bark of *Bombax ceiba* yields tannic and gallic acids having antimicrobial and antioxidant properties. Flowers of *Bombax ceiba* yielded seven flavones, ten flavonoids, Quercetin and more than twenty four other compounds. Most of them have different medicinal properties. Presence of flavonoids and sesquiterpenoids with its free radical scavenging properties are responsible for its antioxidant properties, lipid peroxidation and myeloperoxidase activity. Alkaloids and tannins present in the root stem, bark and leaf of *Bombax ceiba* shows promising activity against various microorganisms such as multi drug resistant *Salmonella typhi*, *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella aerogenes*, *Neisseria gonorrhoeae*, and *Candida albicans*. Aqueous extract shows better antibacterial and antifungal activities. *Bombax ceiba* also shows strong anti- *Helicobacter pylori* activities.

This study has shown the antimicrobial, antioxidant and cytotoxic activities of the root extract of *Bombax ceiba*. The zone of inhibition was tested against *Shigella dysenteriae, Saccharomyces*

cerevisiae and *Pseudomonas aureus*. The number of investigated organism was not enough due to some problems. The zone of inhibitions produced by the crude dichloromethanolic extract of the root of *Bombax ceiba* ranged from 7-10 mm at 300 µg/ disc and 7-8 mm at 600 µg/ disc. The resultant zone of inhibition was not good enough. The test results vary may be due to some experimental error. From the zone of inhibition of Dichloromethane root extract of investigated plant have shown mild to moderate antimicrobial activity. The Dichloromethane root extract has been also showed positive result in antioxidant activity. It has scavenging properties. In Table 4.2, Figure 4.5, the flavonoid content has been shown in both measurement and in curve along with the standard curve. The cytotoxic activity of the Dichloromethane root extract of *Bombax ceiba* was tested by using brine shrimp in proper condition. The brine shrimp was hatched in our laboratory. The standard conditions were to check the result in 8 hours but due to some problems the result was checked after 16 hours. But the result was sufficient and in acceptable level. From the following result that has been shown at Table 4.3, we can conclude that the Dichloromethane root extract of *Bombax ceiba* gives lethal effect in medium concentration.

By using different solvents, different constituents from plants can be isolated. In this study Dichloromethane has been used. Methanol, ethanol, aqueous solutions or other polar and non polar solvents can be used to progress for further research. As *Bombax ceiba* is a medicinal plant enriched with large groups of chemical constituents, further research may lead to develop of an unknown medicine for a known fatal disease.

Chapter 5

CONCLUSION

5.1 Conclusion

The presence of antibacterial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blue print for the development of a drug.

An extensive literature survey has revealed that *Bombax ceiba* has a long history of traditional use for a wide range of diseases. Much of the traditional uses have been validated by scientific research. It is an important species that has economic and ecological importance and should be conserved for ecological perspectives. The plant is used in dysentery, menorrhagia, skin troubles, hemorrhoids, for the treatment of snake bite and scorpion sting, boils, leucorrhoea, internal bleeding, calculus affections, chronic inflammation, ulceration of bladder and kidney, gonorrhea, haemoptysis, influenza, enteritis, pulmonary tuberculosis, cystitis and catarrhal affections bleeding piles. The pharmacological and clinical studies reported in the present review confirm the therapeutic value of *Bombax ceiba*. The presence of interesting/novel chemical compounds indicates that the plant could serve as "lead" for development of novel agents in disorders in the coming years. In this regard, further studies need to be carried out to explore *Bombax ceiba* for its potential in preventing and treating diseases.

The crude dimethanolic extract of *Bombax ceiba* showed significant antimicrobial, antioxidant activities, some of which supports the traditional use of this plant in various diseases. The plant can be a potential source of chemically interesting and biologically important drug candidates. Very few compounds are isolated from the *Bombax ceiba.* Therefore, there is huge potential to find active principles which could be beneficial for mankind for targeting various diseases.

Chapter 6

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