

**Phytochemical & Pharmacological Study of the Extract of the Roots  
of *Bombax ceiba***

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

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## **DECLARATION BY THE CANDIDATE**

I hereby declare that this dissertation, entitled **“Phytochemical & Pharmacological Study of the Extract of the 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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*Dedicated to*  
*My Beloved Parents*

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## ABSTRACT

*Bombax ceiba* has risen the interest of scientists and researchers since some of the active constituents and phytochemicals found on this plant are known to exert many beneficial effects. It is a rich source of phytochemicals, as for example apigenin, a well-known anti-cancer agent, or luteol, triterpene with many health benefits among which its anti-inflammatory, anti-arthritic, anti-mutagenic, antimalarial and anti-plasmodial properties can be mentioned.

The aim of the present study was to find out the phytochemical constituents, evaluate the antimicrobial activity and antioxidant activity of Dichloromethane extract of *Bombax ceiba*.

Preliminary phytochemical screening of roots *B.ceiba* showed the presence of saponins in high concentration, terpenoids in moderate concentration and steroids, flavonoids, carbohydrates, alkaloids, tannins are present in low concentration. The present study indicates that roots of *B.ceiba* possesses significant antibacterial and antioxidant effects.

The antimicrobial activities of dichloromethane solvent extract of plant were tested against the Gram-positive and Gram-negative bacterial strains by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The crude dichloromethane extract of *Bombax ceiba* plant showed poor antimicrobial activities against Gram-positive bacteria and at concentrations of 300 µg/ml and 600 µg/ml per disc.

*In vitro* antioxidant activity test performed by aluminum chloride colorimetric method in which flavonoid concentration was found  $7.8468 \pm 0.6494$  µg/µl equivalent to Quercetin in mg/g. In flavonoid antioxidant test, the absorbance of Dichloromethane extract was taken at 695 nm.

In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and antioxidant properties of *Bombax ceiba*.

**Key Words:** Extract, Dichloromethane, *Bombax ceiba*, phytochemical constituents, Antimicrobial, Antioxidant.

# Chapter 1

## INTRODUCTION

## 1.1 General Introduction

Plants have been used for health and medical purposes for several thousands of years. The number of higher plant species on earth is about 250 000. It is estimated that 35 000 to 70 000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years.

## 1.2 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. In this page, only the flowering plants are mentioned, with little or no references to fungi, ferns, mosses and algae.

Medical herbalism is the practice of healing with medicinal plants. Modern western treatment is different from medical herbalism, but at some point these two merge. For example, the use of friar's balsam or benzoin tincture for treating colds, the use of aloe vera gel for treating sunburn and bruises and the use of cascara or senna to relieve constipation. The tendency in modern medicine is to use synthetic drugs that eventually were modelled on compounds obtained mainly from plants. Therefore, whether the plants are used as a whole, or extracts or their synthetics, their discovery originated from the long term practice of medical herbalism by Man.

### Significances of Medicinal Plants to Human Being

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.

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

#### Plant resources for new medicine

- Bryophytes (nonvascular plants, e.g. liverwort and moss) have about 15,350 species.
- Seedless vascular plants (commonly called fern) are estimated about 12, 157 species
- Gymnosperm has about 760 species. Angiosperm is estimated to have more than 250,000 species.

### **1.3 Medicinal effects of plants developed in Ancient time**

1. Direct test by physicians: for example ancient Chinese physician, Shen Nong tested 70 plant species daily.

2. Lessons from animals: ancient people might gather knowledge of plants for medicinal use on the basis of animal e.g. chimpanzee's self-medication.

- Ethnobotanists

## 1.4 Natural products derived from plants as a source of drugs

Nature, the master of craftsman of molecules created almost an inexhaustible array of molecular entities. It stands as an infinite resource for drug development, novel chemotypes and pharmacophores, and scaffolds for amplification into efficacious drugs for a multitude of disease indications and other valuable bioactive agents.

Since time immemorial, natural products have been the backbone of traditional system of healing throughout the globe, and have also been an integral part of history and culture. Although the use of bioactive natural products as herbal drug preparations dates back hundreds, even thousands, of years ago, their application as isolated and characterized compounds to modern drug discovery and development started only in the 19<sup>th</sup> century. It has been well documented that natural products played critical roles in modern drug development, especially for antibacterial and antitumor agents.

Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidates.

The use of plants as medicines has a long history in the treatment of various diseases. The plant-derived compounds have a long history of clinical use, better patient tolerance and acceptance. To date, 35,000-70,000 plant species have been screened for their medicinal use. Plants especially those with ethnopharmacological uses have been the primary sources of medicine for early drug discovery. Fabricant and Farnsworth, (2001) reported that, 80% of 122 plant derived drugs were related to their original ethnopharmacological purposes. Current drug discovery from plants mainly relied on bioactivity-guided fractionation and led to isolation of many important anticancer drugs such as paclitaxel, camptothecin etc. (Ciddi, 2012).



## 1.5 Medicinal plants in Bangladesh

Bangladesh has very rich in Bio-diversity. It has more than 500 medicinal plants species (Yusuf *et al.*, 1994). An alarmingly populous, but size-wise a very small country is rather unique in having diversified genetic resources in a wide range of habitats. Increasing population pressure and multifarious anthropogenic activities on the natural ecosystems are posing severe and serious threats to once dense and rich genetically diversified plant communities of this country.

Loss of habitats from the wild forests as well as from the village groves, cultivated plains and wild lands is quite common in this country. A broad genetic base has been replaced by a narrow one, and the old genetic diversity is disappearing both inside and outside of the ancient gene centers. This trend is inevitable with the need for highly efficient and uniform cultivars in advanced and sophisticated farming systems.

At present, we have no real protected area for natural genetic resources and also have no specific practical policy on conservation of biodiversity. Although there are several gene banks having limited facilities to preserve some economic crops like rice, jute, wheat, pulses etc in Bangladesh, but there is no centralized organization to maintain germplasms of the wild relatives for agriculture, horticulture, medicinal and economically less important forest species. Bangladesh Agricultural Research Council (BARC) is very worried about this.

However, the rich and diverse heritage of traditional medicinal system in the Indian sub-continent including Bangladesh is increasingly threatened by the interplay of a number of factors such as rapid deforestation and habitat destruction, indiscriminate collection and exploitative trade network.

In Bangladesh there are about 297 Unani, 204 Ayurvedic and 77 Homeopathic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi-processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations. The market value of drugs produced by these industries from medicinal plants is about Tk. 300 crores.

(The Daily Jugantor, 21 June, 2003).

Besides, village Kobiraj, street Vendors and Tribal people also use a large number of medicinal plants for the treatment of various diseases. There is no actual figure how many medicinal plants are used in Bangladesh. Chowdhury at SAARC workshop (held on 16-18 June, 2002) gave a brief idea about the amount of medicinal plants used annually in Bangladesh. A few of them are mentioned here: Ashwagondha (*Withania somnifera*)- 56,000 kg, Anantamul (*Hemidesmus indicus*)- 50,000 kg, Kurchi (*Holarrhena antidysenterica*)-1,00,000 kg, Gulancha (*Tinospora cordifolia*)- 127,000 kg. According to Hamdard Laboratories (WAQF), in Bangladesh the annual demand for a few medicinal plants are- Satomuli (*Asparagus racemosus*)– 800 tons, Sarpagondha (*Rauwolfia serpentina*)– 1,000 tons, Ghritokumari (*Aloe vera*)– 24,000 tons, Kalomegh (*Andrographis paniculata*)– 1,000 tons (Hassan, 2003). Every year Bangladesh imports a large quantity raw materials belonging to of medicinal plants mostly under the banner of spices and spends more than 64 crores Taka annually for this purpose. Ironically, 70% of this imported raw materials can be met from the indigenous sources from Bangladesh.

(Bose et al, 1998).

Medicinal plant species listed by WHO which can be grown in Bangladesh commercially.

Scientific name	Bengali name	English name	Used parts	Used as patent drugs
<i>Winthania somnifera</i> Dunal	Ashwagandha	Winter Cherry	Root, Leaf, Fruit, Seed, whole plant	Syrup Masturin, Arq Ashwaganda, Magun Sohag Soonth
<i>Aloe vera</i> Tour. ex Linn.	Ghritokumari	Aloe	Leaf	Tablet Suranjan, Tablet Mudir, Syrup Belgiri
<i>Andrographis paniculata</i> Wall.ex Nees.	Kalomegh	Creat	Leaf, Stem, whole plant	Syrup Safi, Syrup Kurchi

<i>Asparagus racemosus</i> Willd.	Satomuli	Asparagus	Tuberous root, Leaf, Flower, Fruit	Tablet Abiaj, Khisandha, Ka-4, Sufoof Gigian
<i>Plumbago zeylanica</i> Linn.	Chita		Root	Majoon Falasefa, Syrup Kurchi
<i>Adhatoda zeylanica</i> Nees. (Syn. name- <i>A. vasica</i> Linn.)	Vasak	Vasaka	Leaf, Stem, Bark, Root, Flower	Syrup Saduri, Chawan Prash, Tablet Sualin, Syrup Ajaj
<i>Rauvolfia serpentina</i> (Linn.) Benth.	Swarpagandha	Snake root	Root	Syrup Mangurin
<i>Glycyrrhiza glabra</i> Linn.	Jastimodhu	Liquorice root	Root, Stem	Tablet Sualin, Mauol Hiat, Syrup Badian, Tablet Kafur

(Saleem et al., 1999).

## 1.6 Global status of medicinal plants

Medicinal plants have played a key role in world health. It is estimated that about 25 – 30% of all modern medicines are directly or indirectly derived from higher plants. The herbal products industry comprises a number of inter related sub sectors including as Herbal teas; Functional foods; Nutraceuticals; Phytochemicals; Ethical OTC medicines; Flavours and fragrances; Aroma therapy; Culinary herbs and Spices .

As per World Bank reports trade in medicinal plants, botanical drug products and raw material is growing at an annual growth rate between 5 to 15%. The Global pharmaceutical market has risen from US \$550 billion in 2004 worth to a close to US\$900 billion in the year 2008. The herbal industry shares about US\$62 billion with good growth potential. In India the value of botanicals related trade is about US\$10 billion per annum with annual export of US\$1.1 billion while China annual herbal drugs production is worth US\$48 billion with export of US\$3.6 billion. Presently the United States is the largest market for Indian botanical products accounting for about 50% of the total exports. Japan, Hong Kong, Korea and Singapore are the major importer of the herbal drugs making 66% share of China botanical drug export. Within the European community botanical medicine represents an important share of the pharmaceutical market.

The herbs and botanicals market as it applies to the dietary supplement, self-medication and functional food segments is driven by consumer and health concerns. Broadly speaking, these trends include antiaging, weight control, joint and bone health digestion / immunity cardiovascular health / diabetes, cognition / memory, female / male and the growing wellness and beauty trends. Another trend benefiting the herbs and botanical market is the natural and the exotic ingredients trend, which is taking off in functional food, as well as medicinal products.

(Kumari et al., 2011)

The first commercial pure natural product introduced for therapeutic use is morphine marketed by Merck in 1826, and the first semi-synthetic pure drug aspirin, based on a natural product salicin isolated from *Salix alba*, was introduced by Bayer in 1899. This led to the isolation of early drugs such as cocaine, codeine, digitoxin, quinine and pilocarpine, of which some are still in use and several other recent plant derived compounds, which have undergone development

and have been marketed as drugs which include Paclitaxel from *Taxus brevifolia* for lung, ovarian and breast cancer, Artemisinin from traditional Chinese plant *Artemisia annua* to combat multidrug resistant malaria, Silymarin extracted from the seeds of *Silybum marianum* for the treatment of liver diseases.

However, there is Ayurvedic system of medicine is a traditional system of medicine, native to the Indian subcontinent and practiced in other parts of the world as a form of alternative medicine. In Sanskrit, the word *Ayurveda* consists of the words *ayus*, meaning "life", and *Veda*, meaning "related to knowledge" or "science". Evolving throughout its history, Ayurveda remains an influential system of medicine in South Asia. Over the past centuries, ayurvedic practitioners, called "Ayurvedacharyas," have identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases.

a need to transform Ayurveda into a dynamic, scientifically validated and evidence-based system which takes its roots from rich knowledge base of oral tradition and scriptures. The major hurdle in the wider acceptability of Ayurveda and its products is the lack of proper standardization techniques and its unpreparedness to accept global challenges (Wagner and Farnsworth, 1994). The World Health Organization (WHO) also has emphasized the need to ensure the quality of the medicinal plant products. (Hamberger et al., 1994).

The present study has been performed on *Bombax ceiba* Linn or *Bombax malabaricum* D.C. or *Salmalia malabarica* (DC.) Schott & Endl is belonging to family Bombacaceae (The Wealth of India, 2003). The therapeutic effects have been reported in roots, gums, stem bark, flowers, seeds, prickles and young fruits. The family Bombacaceae consists of 22 genera and 150 species. Genera *Bombax* consists of 60 species, *Ceiba* 15 species, *Durio* 15 species, *Salmalia* 10 species and *Adansonia* 10 species (Rajendra, 2007). This tropical tree has a straight tall trunk and its leaves are deciduous in winter. Red flowers with five 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. (Kirtikar and Basu, 1994).

Reports have shown the presence of glycosides and tannins in roots, stem and leaf. In the stem some alkaloids and in roots, proteins are identified. The stem bark and root contains mangiferin, lupeol and  $\beta$ -sitosterol (Fig. 1). The root bark has 3 naphthalene derivatives related to gossypol (toxic principle of cotton seed) and called as 'semigossypol'. Flowers contain  $\beta$ -sitosterol, traces of essential oil, kaempferol and quercetin. On hydrolysis gums yields arabinose, galactose, galacturonic acid and rhamnose.

It has been claimed in Ayurveda that *Bombax ceiba* possesses proven medicinal properties and is the ingredient of many formulations. The roots are sweet, cooling, stimulant, restorative, astringent, alternative, aphrodisiac, demulcent, emetic and tonic. It is used in the treatment of diarrhea, dysentery and menorrhagia, styptic and for wounds. The gum is cooling, astringent, stimulant, aphrodisiac, tonic and demulcent in nature. It is useful in dysentery, hemoptysis, and pulmonary tuberculosis, influenza, burning sensation, menorrhagia and enteritis. Bark is mucilaginous, demulcent, emetic and tonic. Flowers are astringent and good for skin troubles and hemorrhoids. Seeds are useful in treating gonorrhoea and chronic cystitis. A paste made out of prickles is good for restoring skin color especially on the face. Young fruits are useful in calculus affections, chronic inflammations and ulceration of bladder and kidney.

## 1.7 Aims and objectives

### **Principle aim**

The principle aim of the present study was to investigate the scientific basis of the traditional use of the plant *Bombax ceiba*. To study the other medicinal uses possible and at the same time to find out the chemical groups present in the active plant to get preliminary idea about the active compounds.

## Secondary aim

- ❖ Separation of the plant materials using different solvents.
- ❖ Qualitative analysis of different chemical groups present in the plant extracts.
- ❖ Screening of various pharmacological activities.

## Objectives

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

### 1.8 Performed Tests

Chemical Analysis	Pharmacological Activity Tests
Phytochemical screening:  ➤ Test for Alkaloids, Flavonoids, Tanins, Terpenoids, Saponins, carbohydrates.	➤ Antimicrobial activity test  ➤ Antioxidant activity test.

#### 1.8.1 Phytochemical Screening

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds. Although the knowledge of how these substances provide medicinal value to humans reflects a relatively recent scientific understanding, the use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginnings of medical science.

It is believed that there may be about 4,000 phytochemicals contained in plants that can be used to prevent, minimize or remedy medical conditions such as strokes, cancer or metabolic syndrome. The evidence obtained through current scientific research does not appear to demonstrate that the use of phytochemical supplements supports long-term health as well as consuming the actual fruits, grains and vegetables from which they were taken. The long-term use of phytochemical supplements as a substitute for their natural food sources should only be considered after consulting a doctor, as noted by the American Cancer Society.

### **1.8.2 Antimicrobial activities**

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and “leads” which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. This review attempts to summarize the current status of botanical screening efforts, as well as in vivo studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations.



### 1.8.3 Antioxidant 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, hydroperoxide or lipid peroxy 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.

# **CHAPTER 2**

# **LITERATURE REVIEW**

## 2.1 Diuretic Effects of Young Fruit Extracts of *Bombax Ceiba L.* in Rats

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

(Jalal pure S.et al, 2011)

## 2.2 Ethnobotanical value of dry, fallen ovaries of *Bombax ceiba L.* (Bombacaceae: Malvales)

Indigenous people and their knowledge about nature and natural products have foremost importance in conservation effort. Every community, especially ethnic ones, has strong linkages with plants and the possibility of uncovering new information from these relationships still remain enormous. Ethnobotany which explores human-plant interactions (Pei et al. 2009) is now more important than ever before. Numerous non-timber forest products (NTFPs) have ethnobotanical values on account of their medicinal, food and cultural significance. New uses connected with NTFPs are also being reported and getting documented for posterity. NTFPs constitute an important economic and natural resource, and are used for both family consumption

and commercial trade (Kim et al. 2008). They also meet social needs (Griffiths et al. 2003) and contribute significantly to the livelihood of rural residents (Angelsen & Wunder 2003; Sunderlin et al. 2005). About 80% of the population of developing countries uses NTFPs to meet some of their health and nutritional needs (Beer & McDermott 1996). In many of the thickly populated tropical regions, poor people still collect a wide range of forest products to sustain and supplement their livelihoods and escape hunger and poverty. However, information on such collection efforts and utilization aspects remains unaccounted largely due to the scattered nature of such efforts. (Gopakumar S. et al, 2012)

### **2.3 *In-vitro* Anti-Inflammatory Evaluation of Crude *Bombax ceiba* Extracts**

Plants have contributed lot of medicinal compounds being used today to treat diseases like cancer, hormonal imbalances, jaundice, diabetes, inflammation etc. Medicinal plants are very commonly available in abundance especially in the tropics. They are the vital sources of wide variety of chemicals from which novel anti-inflammatory agents can be discovered. *Bombax ceiba* is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. It is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani. The various parts of *B. ceiba* have been reported for hypotensive and hypoglycemic antiangiogenic, analgesic, antiulcer, antioxidant, hepatoprotective and antimicrobial activities. Also it was used for the treatment of sexual debility, bleeding wounds and vaginal infections. Since there is no scientific report for anti-inflammatory effect of *B. ceiba* bark extract, the present study was an attempt to evaluate the anti-inflammatory activity. (Anandarajagopal K. et al, 2013)

## 2.4 Effect of *B.ceiba* bark extract on Aphrodisiac, birth control, sexual diseases

An ethnobotanical survey of the tribal area of southern Rajasthan was carried out during the year 2001-2002 for ethnosexicological herbal medicines. *B. ceiba* was used as described: half a cup of ethanol extract of bark and powder was given for 3 days to both men and women with sexual diseases like hydrocele, leucorrhoea, gonorrhoea and was also used to check menstrual disorders in women.

Studies on the ethnomedicobotany of the Kandha tribe of Orissa showed that one teaspoon juice of fresh stem bark of *B. ceiba*, one teaspoon juice of fresh root of *Asparagus racemosus*, powder of seven black peppers (dried seed of *Piper nigrum* L., Piperaceae) and one teaspoon of processed sugar or gum taken orally on an empty stomach two times daily for 21 days to cure gonorrhoea, impotency, spermatorrhea, sterility, nocturnal emission and leucorrhoea. It is also prescribed for increasing sperm in semen and to act as aphrodisiac. (Manu Vhokta 2014).

## 2.5 Free Radical Scavenging Property of *Bombax ceiba* Linn. Root

Silk cotton tree (*Bombax ceiba* Linn.) is a well-known ethnomedicinal plant. Root of this plant was investigated for its antioxidant potential for the first time. Assessment of antioxidant activity was done using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and reducing power assay. Preliminary phytochemical screening of the roots showed presence of phenolics, tannins, flavonoids, steroids, saponins and cardiac glycosides. Methanolic extract of the roots showed high amount of phenolics (30.95% w/w) and tannins (15.45% w/w) and a very good DPPH radical scavenging activity ( $EC_{50}$  of 15.07  $\mu$ g) in a dose dependent manner. The extract showed dose-dependent reduction ability ( $Fe^{3+}$  to  $Fe^{2+}$  transformation) with a maximum absorbance of 1.11 at a concentration of 500  $\mu$ g of the extract. Acute study in healthy human volunteers showed a significant ( $p < 0.05$ ) rise in total antioxidant status at the end of 4 h after administration of 3 g root powder. This strong *in vitro* and *in vivo* antioxidant potential

of *B. ceiba* dry root powder validates its uses in diabetes mellitus and heart disease as described in the traditional medicine. (Vain J. et al; 2011)

## 2.6 Genetic Divergence in *Bombax ceiba* L. Germplasms

Thirty germplasm lines of *Bombax ceiba* were evaluated for ten traits to study the pattern of genetic divergence using MAHALANOBIS' D2 statistics. The genotypes were grouped in ten clusters. Cluster II, III and IV showed greater divergence. Cluster II was the most distant from others. Clusters mean indicated that cluster III was the best for plant height, stem girth (diameter), primary and secondary branches, root length and stem biomass, while cluster II and IV was the best for root width, leaf biomass and root biomass, respectively. Based on mean performance, genetic distance and clustering pattern hybridization involving germplasm lines, belonging to cluster II, III and IV, respectively; as parents for intra-population improvement may pave way for enhancing variability so as to select progenies with greater plant height, stem diameter and improved timber quality.

(Chaturvedi, 2001)

## 2.7 Pollination biology in *Bombax ceiba* Linn.

A study of flower morphology, anthesis, pollen production, foraging nature of flower visitors, in vitro pollen germination and stigma receptivity of *Bombax ceiba* Linn. of the family Bombacaceae has been made. The flowers are large with numerous stamens which open in post middle night and continue up to the morning. Anther dehisced after flower opening. During daytime, different types of birds visit the flowers and subsequently help in pollen dispersal and pollination when stigmas remain receptive. Each flower produced 8,863,000 pollen grains which are of 3-colporate, with reticulate ornamentation and thick exine. In vitro pollen germination study indicated that best germination (97%) along with 2940  $\mu\text{m}$  tube development, takes place in 20% sucrose combined with 500  $\mu\text{g/ml}$   $\text{H}_3\text{BO}_3$  solution. Among different salts of Ca, Mg and K, only  $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$  showed significant result with 54% germinating pollen along with 420  $\mu\text{m}$  tube length in 50  $\mu\text{g/ml}$   $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$  solution. Maximum stigma receptivity was noticed during the first day after anthesis with 61% in vivo germinating pollen captured with unicellular pointed papillae cells over receptive stigma surface. Atmospheric pollen frequency was found to be 5.17% in 10.00 h. No fruit setting was observed in netted and bagged flowers, which strongly indicates that some external agents are required for successful pollination.

(Ashoke, 2000)

# **CHAPTER 3**

## **MATERIALS & METHODS**



### 3.1 Phytochemical Investigation

Presence of different chemical groups present in the extract represents the preliminary phytochemical screening. For phytochemical screening, 20 ml of the DICOLORO METHANE extracts of *Bombax ceiba* were used before it was evaporated. The extracts were transferred in 50 ml beakers respectively using pipettes for the extracts with proper labeling. Then 10 clean test tubes were in taken in two test tube racks and labeled with the name of the extracts and test names to prevent confusion. Phytochemical screening was performed using standard procedures and specific reagents were used for the different chemical group test.

(Mojab F.et al, 2003)

### 3.2 Apparatus and instruments

Test tube                      micropipette

Watch glass                 Beaker

Holder                        Dropper

Filter paper

Funnel

Conical flask

#### **Test compounds**

Dichloromethane extracts of *Bombax ceiba*.

### 3.3 Methods of different phytochemical test

#### Tests for carbohydrates

a) Benedict's Test: To 0.5ml of filtrate, 1ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 5 min. a characteristic colored ppt. indicates the presence of sugar.

b) Barfoed's Test: To 1ml of extract, 3ml of Barfoed's reagent is added & heated on a water bath for 2 min. Red ppt. indicates presence of sugar.

#### Identification

- All carbohydrates should give a positive reaction. Monosaccharide gives a rapid positive test where disaccharide reacts slowly with Molish's test.
- Either of the aldehydes if present will condense with two molecules of naphthol to form a purple color product.

#### Tests for alkaloid

A small volume of each extract was neutralized by adding 1 or 2 drops of dilute  $H_2SO_4$ . This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation was observed:

Mayer's reagent: Formation of white and cream color precipitate indicated the presence of alkaloids.

Hager's reagent: Formation of yellow crystalline precipitate indicated the presence of alkaloids.

Wagner's reagent: Formation of brownish-black ppt. indicated the presence of alkaloids.

## Identification

1. Creamish, brownish-red or orange ppt. indicated the presence of alkaloids.
2. A sign (+) denotes low concentration if addition of the reagent produce a faint turbidity.
3. A sign (++) denotes moderate concentration if the addition of reagent produce light opalescence precipitate.
4. A sign denotes high concentration if the addition of reagent produce yellowish-white precipitate.

## Test for saponin

About 1ml of extract was added to 10ml of distilled water, production of foam with the addition of olive oil indicates the evidence of saponins.

Identification:

1. A sign (+) denotes low concentration of saponins when the forth reached a height of 0.5cm.
2. A sign (++) denotes moderate concentration of saponins when the forth reached a height of 0.6-1cm.
3. A sign (+++) denotes high concentration of saponins when the forth reached a height of more than 1cm.

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

## Identification

1. An immediate red coloration indicates the presence of flavonoids.
2. The yellow coloration disappeared on standing.
3. A sign (+) denotes low concentration of flavonoids if pale yellow color was observed.

4. A sign (++) denotes moderate concentration of flavonoids if moderate color was observed.
5. A sign (+++) denotes high concentration of flavonoids if high color was observed.

**Test for steroids:** A small amount of extract was added with 2 ml of chloroform, then 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> was carefully added from the side of the test tube. In presence of steroids, a red color was produced in chloroform layer.

### Identification

Reddish brown colored ring forms in some samples indicating the presence of steroids.

**Test for tannins:** About 0.5 ml of extract was stirred with 10 ml of distilled water. Production of a blue, blue-black, green or blue-green coloration or precipitation on the addition of FeCl<sub>3</sub> (5%) reagent was taken as evidence for the presence of tannins.

### Identification

1. A sign (+) denotes low concentration of tannins when a slight precipitate was observed.
2. A sign (++) denotes moderate concentration of tannins when a medium precipitate was observed.
3. A sign (+++) denotes high concentration of tannins when a heavy precipitate was observed

**Test for terpenoids:** About 2ml dichloromethane was added to the sample, then conc. H<sub>2</sub>SO<sub>4</sub> was carefully added, reddish brown coloration is the evidence for the presence of tarpenoids.

### Identification:

1. Reddish-brown coloration at the interface indicates the presence of terpenoids.
2. A sign (+) denotes when a faint reddish brown coloration was observed.
3. A sign (++) denotes when a medium reddish brown coloration was observed.
4. A sign (+++) denotes when a deep reddish brown coloration was observed.

### 3.4 *In vitro* Determination of the Antioxidant Activities

The antioxidant activity of the Dichloromethane extracts of *B.Ceiba* was determined by using the following methods.

#### ❖ 3.5 Determination of Flavonoids Content

##### **Principle**

Aluminum chloride colorimetric method was used for flavonoids determination. 1.5 ml extract (1mg/ml) in methanol was taken. After that, 6ml distilled water was added with 0.45 ml Sodium Nitrate (5%w/v).The mixture was incubated for 6 minutes, then 0.45 ml Aluminum chloride(10%) taken and again incubated for 6 minutes. Then, 6 ml Sodium hydroxide (4%) was taken and 0.6ml distilled water added. The absorbance of the reaction mixture was measured at 510 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100ml of sample.

Concentration of flavonoids was expressed in terms of mg/100ml of sample.

## Reagent

Aluminum Chloride ( $\text{AlCl}_3$ )

Sodium Nitrate

Sodium Hydroxide

Methanol

Quercetin (Analytical or Reagent grade)

## Instruments and apparatus

Electronic balance

Funnel

Test tubes

Micropipette

Incubator

UV- spectrophotometer

## Procedure

### Preparation of 10% Aluminum chloride ( $\text{AlCl}_3$ ) solution

10 gm. of  $\text{AlCl}_3$  was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

### Preparation of 5 % Sodium Nitrite ( $\text{NaNO}_2$ ) solution

5 gm. of  $\text{NaNO}_2$  was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

### Preparation of Standard solution

Quercetin stock solution of concentration  $5\mu\text{g}/\mu\text{l}$  was prepared by dissolving 0.025 gm of quercetin into 5 ml of methanol. The experimental concentrations from the stock solution were prepared by the following manner:

Concentration ( $\mu\text{g}/\text{ml}$ )	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled methanol	Final volume
100	100 $\mu\text{l}$	-	4.90 ml	5 ml
50	-	2 ml (100 $\mu\text{g}/\text{ml}$ )	2 ml	4 ml
25	-	2 ml (50 $\mu\text{g}/\text{ml}$ )	2 ml	4 ml
12.5	-	2 ml (25 $\mu\text{g}/\text{ml}$ )	2 ml	4 ml

### Preparation of Extract solution

0.050 gm of each plant extracts were taken and dissolved into 5 ml of methanol. The concentration of the solution was  $10\mu\text{g}/\mu\text{l}$  of plant extracts.

## Experimental procedure

1.5 ml extract (1 mg/ml) was taken in methanol



6 ml distilled water added.



0.45 ml NaNO<sub>2</sub> (5% w/v) taken.



6 minutes incubation.



0.45 ml AlCl<sub>3</sub> (10%) taken and again incubated for 6 minutes.



6ml NaOH (4%) taken with 0.6 ml distilled water and incubate dfor 15 minutes.



Measuring absorption at 695 nm.



### 3.6 *In vitro* Antibacterial Screening

#### **Antibacterial screening**

Bacteria is 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) Bio autographic method

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

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

### **Test materials used for this study**

- Dichloromethane crude extracts of *Bombax ceiba* for the investigation of antibacterial activity.
- Solvent (methanol) were used to dissolve the compounds.
- Standard disc.

### **Apparatus and Reagents**

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

## Test organisms

Gram Positive	Gram negative
<i>Staphylococcus aureus</i>	<i>Vibrio parahemolyticus</i>
	<i>Vibrio mimicus</i>
	<i>Escherichia coli</i>

### 3.7 Culture medium and their composition

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

#### Nutrient agar medium

Ingredients	Amounts
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm

Distilled water q.s. to 100 ml

p<sup>H</sup> 7.2 ± 0.1 at 25<sup>0</sup>C

### **Nutrient broth medium**

Ingredients	Amounts
-------------	---------

Bacto beef extract	0.3 gm
--------------------	--------

Bacto peptone	0.5 gm
---------------	--------

Distilled water q.s.to	100 ml
------------------------	--------

p<sup>H</sup> 7.2 ± 0.1 at 25<sup>0</sup>C

### **Muller – Hunton medium**

Ingredients	Amounts
-------------	---------

Beef infusion	30 gm
---------------	-------

Casamino acid	1.75 gm
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<b><i>Starch</i></b>	<b><i>0.15 gm</i></b>
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Bacto agar	1.70 gm
------------	---------

Distilled water q.s. to 100 ml

p<sup>H</sup> 7.3 ± 0.2 at 25<sup>0</sup> C

### **Tryptic soya broth medium (TSB)**

Ingredients	Amounts
Bacto tryptone	1.7 gm
Bacto soytone	0.3 gm
Bacto dextrose	0.25 gm
Sodium chloride	0.5 gm
Dipotassium hydrogen Phosphate	0.25 gm
Distilled water q.s. to	100 ml
pH	7.3 ± 0.2 at 25°C

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

#### **3.7.1 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<sup>H</sup> (at 25 °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 °C for 20 minutes. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

### **3.7.2 Sterilization procedure**

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

### **3.7.3 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 °C for their optimum growth. These fresh cultures were used for the sensitivity test.

### **3.8 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 petridishes. The petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

### 3.8.1 Preparation of Disc

#### **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.

#### **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.

### 3.8.2 Preparation of sample disc

Dichloromethane extracts of *Bombax ceiba* were tested for antimicrobial activity against a number of both gram positive and gram negative bacteria the amount of sample per disc was 300 µg and 600 µg.

#### 3.8.2.1 Preparation and application of the test samples

Sample discs were prepared by adding 300 µl and 600 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.

### 3.8.2.2 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 °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°C for 24 hours.

### 3.9 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.



Fig: Zone of inhibition

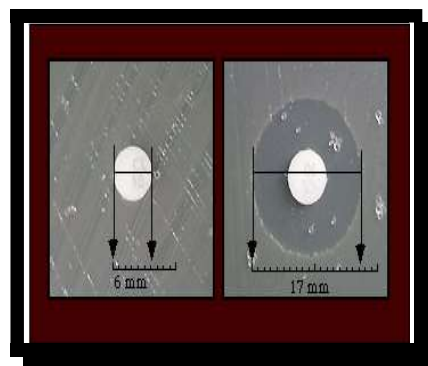


Fig: Measuring Zone of Inhibition



# **CHAPTER 4**

**RESULT**

**&**

**DISCUSSION**

#### 4.1 Results of Phytochemical analysis

Qualitative analysis of the Phytochemicals of *Bombax ceiba*

Plant extract	Alkaloid Test	Carbohydrate Test	Steroid test	Tannin test	Saponin Test	Terpenoid test	Flavonoid Test
Dichloromethane extract of <i>Bombax ceiba</i>	+	+	+	+	+++	++	+

Symbol (+++) indicates presence in high concentration, Symbol (++) indicates presence in moderate concentration, Symbol (+) indicates presence in low concentration of the respective phytochemicals.

## 4.2 Result of Antibacterial Screening

The extract of Dichloromethane was active against all the test organisms. The Dichloromethane extract (300 µg/disc, 600µg/disc) of *Bombax ceiba* showed antibacterial activity.

Name of the test organisms	Diameter of the zone of inhibition (in mm)		
	Dicholoromethane extract (300 µg/disc)	Dicholoromethane extract (600 µg/disc)	Kanamycin disc (30 µg/disc)
<b>Gram positive</b>			
<i>Staphylococcus aureus</i>	-	-	34 mm
<b>Gram negative</b>			
<i>Vibrio mimicus</i>	9 mm	8 mm	20 mm
<i>Vibrio parahemolyticus</i>	7 mm	8 mm	30 mm
<i>Escherichia coli</i>	7 mm	-	13 mm

### 4.3 Result of Antioxidant activity by Flavonoid method

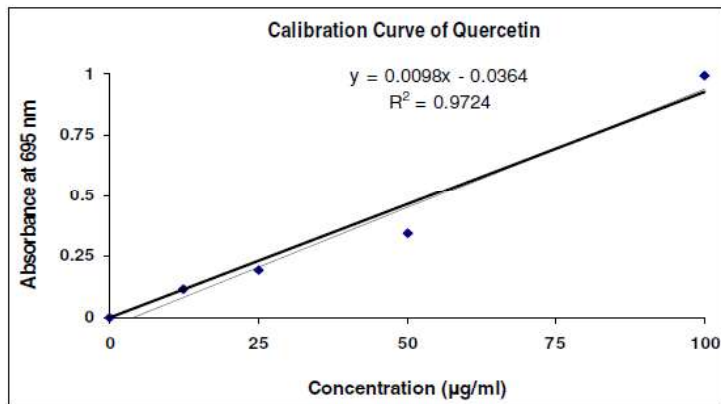


Figure 4.4: Calibration curve of quercetin.

Absorbance	Equation	Value of X	(Amount of total flavonoid content equivalent to Quercetin in mg/g) ± std. deviation
0.045	$Y = 0.0098x - 0.0364$	8.306 µg/µl	7.8468 ± 0.6498 µg/µl
0.036		7.387 µg/µl	

#### 4.4 Discussion

Preliminary phytochemical screening of roots *B.ceiba* showed the presence of steroids, flavonoids, terpenoids, carbohydrates, alkaloids, tannins, saponins, The present study indicates that roots of *B.ceiba* possesses significant antibacterial and antioxidant effects.

The antimicrobial screening using a sensitive *in vitro* disc diffusion method & the extract of dichloromethane was active against all most all the test organisms. The dichloromethane extract ((300 µg/disc & 600 µg/disc) of *B.ceiba* showed antibacterial activity only against Gram negative bacteria. This result suggests that *B.ceiba* can be a potential source of antimicrobial agents against gram negative bacteria.

Plants rich in secondary metabolites, including phenolics, flavonoids and carotenoids, have antioxidant activity due to their redox properties and chemical structures. Subsequently, Antioxidant activity plays very important role to cure diseases and also for health promotion. The screening of plant extracts and natural products for antioxidant and antimicrobial activity has revealed the potential of higher plants as a source of new agents to serve the processing of natural products. (Mokbel and Suganuma, 2006).

Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. (Sharififer et al., 2008).

The antioxidant effect of the roots of *B.ceiba* was determined by Aluminum chloride colorimetric method measuring the change in absorbance at 695 nm by UV- spectrophotometer. The flavonoid content is responsible for the bioactivity of these crude extracts. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defense. Thus, the antioxidant property of *B.ceiba* would produce an additional therapeutic benefit enhancing its anti-inflammatory.

Since a variety of constituents is present in the extract studied, it becomes difficult to describe the antioxidant properties selectively to any one group of constituents without further studies, which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active antioxidative principles present in this plant.

# **CHAPTER 5**

# **CONCLUSION**

## 5.1 CONCLUSION

The present in-vitro study is a preliminary evaluation of phytochemical screening, anti-microbial activity and anti-oxidant of *B. ceiba* demonstrated that *B. ceiba* can be used to inhibit bacterial growth and cure the inflammation. In case of anticancer drug preparation this plant extract can be a good target. The antimicrobial activity of the plant extracts were tested against four potentially microorganisms by using disc diffusion method at different concentrations of the Dichloromethane extracts of *B.ceiba* to understand the most effective activity. It has god antibacterial effect. The Dichloromethane extracts of *B.ceiba* showed moderate antioxidant effect. The anti-oxidant property depends upon concentration and increased with increasing amount of the Dichloromethane extract in all models.

This study is a preliminary study but the root of the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. Isolation of the respective phyto constituents from *B. ceiba* directs to investigate the possible mechanism of action at cellular level which may become a useful approach to develop natural bioactive products. The present study suggests that roots of *B. ceiba* would serve as a source for the discovery of novel therapeutic agents.



# CHAPTER 6

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