A Study of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* Leaves

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:

Noshin Sharmili

ID: 2013-1-70-061

Department of Pharmacy

East West University

Department of Pharmacy

DECLARATION BY THE CANDIDATE

I, **Noshin Sharmili,** hereby declare that this dissertation entitled **"Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* Leaves"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine and authentic research work carried out by me. The content of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma or Fellowship.

Noshin Sharmili

ID: 2013-1-70-061

Department of Pharmacy

East West University

Aftabnagar, Dhaka

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation entitled, **"Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* Leaves"** is a research work carried out by, **Noshin Sharmili** (ID: 2013-1-70-061) in 2017, under the supervision and guidance of me, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy. The thesis has not formed on the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

Nazia Hoque

Assistant Professor

Department of Pharmacy,

East West University, Dhaka

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation entitled, **"Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* Leaves"** is a research work carried out by **Noshin Sharmili** (ID: 2013-1-70-061), under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

Dr. Chowdhury Faiz Hossain

Chairperson and Professor

Department of Pharmacy

East West University

Aftabnagar, Dhaka.

ACKNOWLEDGEMENTS

To begin with, I would like to remember the mercy and kindness of Almighty Allah for completing my research work appropriately. It is Him who has sustained me throughout the course of my study.

I would like to express my sincere thanks to East West University for giving me the opportunity and for seeing sufficient merit in me to conduct this study in the East West University laboratory of the Department of Pharmacy.

I would like to express my respect, cordial gratitude and obligation to my thesis supervisor, **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University for her scholarly guidance, constant supervision and support related to the research work and completion of the thesis paper from the very beginning to the end.

It is also a great pleasure for me to offer my sincere regards and profound gratitude to **Dr**. **Chowdhury Faiz Hossain**, Professor & Chairperson and **Dr. Shamsun Nahar Khan**, Associate Professor, Department of Pharmacy, East West University for giving me necessary support and for facilitating a smooth condition for my study.

I am thankful to the laboratory officers of the Department of Pharmacy, East West University, for their assistance in the laboratory.

I am especially grateful to my parents for their help, sacrifices, cooperation and inspirations in my work. Last but not the least, I owe special thanks to my fellow group members for being with me in this journey and supporting me in my research work.

Thank You.

Dedication

This Research Paper is dedicated to my beloved parents for their unconditional support.

ABSTRACT

Many pharmaceutical drug discoveries originated from traditional folk medicine and it's associated plant materials and bioactive secondary metabolites. The genus *Garcinia,* belonging to the Family Clusiaceae which comprises of about 300 species, have been widely investigated in terms of their bioactive ingredients. *Garcinia* is a rich source of secondary metabolites. Phytochemical investigations of the plant parts indicated that the fruit, twig and stem are the best source of secondary metabolites, providing flavonoids, phloroglucinols and xanthones respectively. Many of the isolated compounds have a wide range of pharmacological activities including anticancer, anti-inflammatory, antibacterial, antiviral, anti-fungal, anti-HIV, antidepressant and antioxidant.

The aim of the present study was to evaluate the antioxidant and antimicrobial activity of dichloromethane extract of *Garcinia cowa.* The antioxidant activity was measured by DPPH and total Phenol test. The IC_{50} values of DPPH test was 51.7964 μg/ml for dichloromethane extract of *G.cowa* leaves. The Total Phenol content was 173.93±5.78 mg/g equivalent to Gallic Acid for methanol extract of *Garcinia cowa* leaves. By determining antioxidant property, the result suggests that the plant extract which have been tested possesses antioxidant property. The antimicrobial activities of dichloromethane extract of *Garcinia cowa* leaves were tested against ten microorganisms by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The dichloromethane extract of *Garcinia cowa* leaves showed good antimicrobial activities (11 mm-23 mm) against the microorganisms. Dichloromethane extract of *Garcinia cowa* leaves showed highest activity against *Salmonella paratyphi* and moderate activity against *Bacillus sereus, Vibrio parahemolyticus* and *E.coli.* No activity was found agaisnst *Bacillus megaterium, Staphylococcus aureus, Shigella dysenteriae* and *Pseudomonas aureus.*

In conclusion, further investigations are needed to identify the active constituents and the exact mechanisms of action responsible for the reported antioxidant and antimicrobial properties of *Garcinia cowa.*

Key Words: Medicinal Plant, *Garcinia cowa*, Secondary Metabolites, DPPH, Antioxidant, Antimicrobial, Total Phenol

Lists of Contents

Chapter 1: Introduction

Serial No.	Topic	Page No.
1.1	Introduction	1-2
1.2	Medicinal Plants	2-3
1.3	Plants as a basis of some important drugs	3
1.4	History of the use of plants as medicines	4-5
1.5	Traditional medicine	5
1.6	Antioxidants	6
1.7	Classification of antioxidants	6
1.7.1	Natural antioxidants	6
1.7.1.1	Enzymatic antioxidants	7
1.7.1.2	Primary antioxidants	7
1.7.2	Secondary antioxidants	7
1.7.2.1	Non enzymatic antioxidants	8
1.7.2.2	Minerals	8
1.7.2.3	Iron (Fe)	8
1.7.2.4	Magnesium (Mg)	8
1.7.2.5	Selenium (Se)	8
1.7.2.6	Vitamins	9
1.7.2.6.1	Vitamin A	9
1.7.2.6.2	Vitamin C	9
1.7.2.6.3	Vitamin E	9
1.7.2.7	Carotenoid	10
1.7.2.8	Polyphenols	10
1.8	Other antioxidants	11
1.8.1	Bilirubin	11

1.8.2	Uric acid	11
1.8.3	Coenzyme Q	11
1.9	Characteristics of medicinal plants	12
1.9.1	Synergistic medicine	12
1.9.2	Support of official medicine	12
1.9.3	Preventive medicine	12
1.10	Importance of medicinal plants	12-14
1.11	Some common medicinal plants in Bangladesh	14-15
1.12	Families of medicinal plants	15-24
1.13	Review on mangosteen (*Garcinia cowa*)	24
1.14	General Information	25
1.15	Taxonomical classification	25
1.16	Distribution	25
1.17	Parts used	25
1.18	General description	25
1.19	Mode of uses	26-27
1.20	Salient features of family Clusiaceae	27-28
1.21	Chemical constituents & biological activities of *Garcinia cowa*	28-31
1.22	Distribution and biological activity	31
1.23	Classes of compounds isolated from *Garcinia cowa*	31
1.23.1	Depsidone	31
1.23.2	Flavonoids	32-33
1.23.3	Xanthones	33
1.24	Antibacterial activity	33
1.25	Anti-inflammatory activity	33-34

Chapter 2: Literature Review

Serial No.	Topic	Page No.
2.1	Literature review on *Garcinia cowa*	35
2.1.1	Antioxidant and antiplatelet aggregation properties of the bark extracts of *Garcinia pedunculata* and *Garcinia cowa*	35
2.1.2	Cowaxanthone F and other anti-inflammmatory and antioxidant compounds from *Garcinia cowa*	35
2.1.3	Cytotoxic compounds from the leaves of *Garcinia cowa*	35-36
2.1.4	Organic acids from leaves, fruits and rinds of *Garcinia cowa*	36
2.1.5	Updates on antiobesity effect of *Garcinia* origin (-)-HCA (Hydroxycitric acid)	36-38
2.1.6	Cytotoxic and nitric acid inhibitory activities of methanol extracts of *Garcinia* species	38-39
2.1.7	Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria	39

Chapter 3: Methodology

Serial No.	Topic	Page No.
3.1	Theory of phytochemical screening	40
3.1.1	Materials (Reagents and Tools) used	40
3.1.2	Test compounds	40
3.1.3	Preparation of sample solution	41
3.1.4	Phytochemical tests	41-42
3.2	Assessment of in vitro pharmacological property	42
3.2.1	Determination of antioxidant property	42
3.2.2	DPPH free radical scavenging activity	42
3.2.2.1	Principle	42

3.2.2.2	DPPH solution	43
3.2.2.3	Preparation of standard/extract solution	43
3.2.2.4	Procedure	44
3.2.2.5	Calculation	45
3.3	Determination of total phenolic content	45
3.3.1	Principle	45
3.3.2	Preparation of 7.5% sodium carbonate solution	46
3.3.3	Preparation of standard solution	46
3.3.4	Preparation of extract solution	46
3.3.4.1	Experimental procedure	47
3.4	Antimicrobial screening	48
3.4.1	Materials	48
3.4.1.1	Microorganisms	48
3.4.1.2	Test organisms	48
3.4.1.3	Culture media and chemicals	48
3.4.1.4	Equipment	49
3.4.1.5	Test materials	49
3.5	Methods	49
3.5.1	Culture preparation	49
3.5.1.1	Composition of culture media	49
3.5.1.2	Composition of nutrient agar media (1000 ml)	49
3.5.1.3	Sterilization procedure	50
3.5.1.4	Preparation of subculture	50
3.5.1.5	Preparation of test plates	50
3.6	Preparation of discs	50
3.6.1	Standard discs	50-51
3.6.2	Blank discs	51
3.6.3	Preparation of sample discs with test samples	51

3.6.4	Placement of disc and incubation	51
3.6.5	Determination of zone of inhibition	51

Chapter 4: Results and Discussion

Serial No.	Topic	Page No.
4.1	Phytochemical screening of dichloromethane extract of *Garcinia cowa* leaves	52
4.2	DPPH test of dichloromethane extract of *Garcinia cowa* leaves	52
4.2.1	Results of DPPH test of dichloromethane extract of *Garcinia cowa* leaves	53
4.3	Total phenol content of dichloromethane extract of *Garcinia cowa* leaves	54
4.3.1	Preparation of standard curve for gallic acid	54
4.3.2	Results of total phenol content	54
4.4	Antimicrobial screening of dichloromethane extract of *Garcinia cowa* leaves	55
4.5	Discussion	56-57

Chapter 5: Conclusion

Serial No.	Topic	Page No.
5	Conclusion	58

Chapter 6:

References……

List of Figure

Figure No.	Topic	Page No.
1	Whole plant of *Garcinia cowa*	28
2	Flower of *Garcinia cowa*	29
3	Fruit of *Garcinia cowa*	30
4	Foliage of *Garcinia cowa*	30
5	DPPH scavenging activity of dichloromethane extract of *Garcinia cowa* leaves	53
6	Standard curve of gallic acid	54

List of Table

Table No.	Topic	Page No.
1	Common medicinal plants in Bangladesh	14-15
2	Reagents used in phytochemical screening	40
3	Reagents used in DPPH free radical scavenging activity	43
4	Amount of standard or extract solution	44
5	Reagents used in the determination of total phenolic content	45
6	Concentrations used in the preparation of standard solution	46
7	Concentrations used in the preparation of extract solution	47
8	List of test bacteria	48
9	Composition of nutrient agar media	49
10	Result of phytochemical screening of dichloromethane extract of *Garcinia cowa* leaves	52
11	Result of absorbance and % of inhibition of dichloromethane extract of *Garcinia cowa* leaves and ascorbic acid	52
12	Result of DPPH test of dichloromethane extract of	53

	Garcinia cowa leaves	
13	Result of total phenol content of dichloromethane extract of *Garcinia cowa* leaves	54-55
14	Result of zone of inhibition of dichloromethane extract of *Garcinia cowa* leaves and Kanamycin	55

Chapter 1

Introduction

1.1 Introduction:

There is real evidence that humans have relied on Nature for medicines for thousands of years and plants or plant extracts have formed the basis of traditional medicine. Traditional medicine is still practiced nowadays in different parts of the world and was the origin of medical treatments. Even though the use of plants as medicinal drugs is an ancient science which dates back several millennia, many of the most significant drug advances have been made within the last century. It would be reasonable to state that man's encounter with sickness has prompted the rubbing or chewing of seeds, leaves, and other parts of plants in order to alleviate illness. Historical trial and error practices on different plants resulted in some plants being helpful against diseases and, gradually over time, specific plants were discovered that treat certain diseases.

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life. (Sofowora, 1982).

Plants have been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes.

In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their

own version of plant compounds and, over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals.

Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. In Germany, about 600 to 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use. (Ghani, 1998).

1.2 Medicinal Plants:

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. When a plant is designated as "medicinal", it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes. There are a huge number of medicinal plants. In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of the world's flowering plant species have been used medicinally. Sometimes the figure of 70,000 medicinal plant species is cited, but this includes many algae, fungi, and micro-organisms that are not really plants as the word is understood by botanists. In any event, there is no other category of plants useful to man (with the possible exception of ornamental plants) that includes so many species, and the question naturally arises why such a staggering number of plants have useful medicinal properties. 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. 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.

The association of humans and animals with plants obviously originated with the beginning of life on earth, when plants supplied much of the shelter, oxygen, food and medicine needed

by higher life forms. Overtime and with the beginning of societies, human learned to recognize and categorize plant materials suited for use in meeting the necessities of life. Of these necessities, the use of herbs and herbal extracts for their healing powers can be traced to earliest of myths, traditions and writings used to codify those plants that can ease pain and treat diseases. The evolution of these plant-based medicine systems, primarily based on plants within a local area, produced the well-known traditional medicine systems, the Ayurvedic and Unani of the Indian subcontinent, the Chinese and Tibetan of other parts of Asia, the Native American of North America, the Amazonian of South America and several local systems within Africa. According to World Health Organization (WHO), about 70 percent of the world's population relies on plants for their primary health care and some 35,000 to 70,000 species has been used as medicaments, a figure corresponding to 14-28% of the 250,000 plants species estimated to occur around the world, and equivalent to 35-70% of all species used world-wide. In today's global market, more than 50 major drugs originated from tropical plants. From about 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential. The chemical and biological diversity of plants represent a potentially limitless renewable source for the use in the development of new pharmaceuticals. (Sofowora, 1982).

1.3 Plants as a Basis of Some Important Drugs:

Higher plants have been used as a source of drugs by mankind for several thousand years. In fact, ancient man was totally dependent on green plants for his day-to-day needs of medicaments. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. With the advancement of synthetic organic chemistry most of the active constituents of plants used in medicine were synthesized. At one time it was thought that ultimately all the plant drugs would be obtained from synthetic sources. However, in spite of phenomenal progress in the development of new drugs from synthetic sources and the appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw materials for some of the most important drugs. Although data are not available for all countries, a study carried out in the United States by Farnsworth and his colleagues between 1958 and 1980 indicated that although the number of prescriptions issued by community pharmacies in the United States increased considerably, the percentage of prescriptions containing one or more plant products

remained constant at a figure of 25%. It has been found that in highly developed countries like the United States more than 100 chemical constituents of definite structure derived from 41 species of plants were used in modern medicine. It has also been estimated that in addition to these active constituents, more than 96 crude extracts were also used in the United States. (Faried, 2000).

1.4 History of the Use of Plants as Medicines:

One of the oldest records and considered the oldest written evidence of medicinal plants usage goes back to about 5000 years ago and was written on Sumerian clay slabs containing a dozen recipes for drug preparations of over 200 different plants. Other records, which date back to around 2600 BC, came from ancient Mesopotamia and were recorded on clay tablets in cuneiform script. There is evidence that medicinal plants have been used in early civilizations demonstrated by the Chinese emperor Shen Nung (ca. 2500 BC) who compiled descriptions of over 300 medicinal herbs and the Chinese Materia Medica has a long impressive history. Many of those herbs have been reported as medicines in different parts of the world such as ancient Egypt, Mesopotamia, and Europe. Opium is one of mankind's oldest effective drugs and was used several millennia ago. The Assyrians, Babylonians, and Sumerians recorded herbal remedies in cuneiform inscriptions on numerous clay tablets and the Code of Hammurabi (18th century BC) contains many herbal medicines. The Egyptians recorded their medicinal knowledge in tomb illustrations and on papyrus dating from the Old Kingdom of Egypt. The 'Ebers Papyrus' (ca. 1550) was the most important of these recordings and contains over 600 prescription drugs of various plant species. European medicine is thought to have initiated with Hippocrates (460–377 BC) who compiled over 200 medicinal plants which were classified by physiological action and he is considered the 'father of medicine'. Celsus (25 BC– 50 AD), who was a famous medical writer, mentioned about 250 medicinal plants in his 'De re medica' book. Dioscorides, who was a Greek military physician and considered the 'father of pharmacognosy', recorded the use of medicinal plants and wrote De Materia Medica in ca. 77 AD, which was used as a reference in Europe for more than a millennium and translated into several languages. It included over 900 drugs and most of them were of plant origin. Galen (130–200AD), who was a renowned Greek physician and pharmacist, influenced the development of various scientific disciplines and documented the use of plants as medicines.

Native Americans used plants as medicines for centuries and some anticancer drugs that are currently available to treat various cancers are derived from plants in North America such as the Pacific yew tree (*Taxus brevifolia*) from which the anticancer drug paclitaxel (Taxol® , 1) is derived .African traditional medicine has also been practiced for many centuries and it has diverse medical treatments for different diseases but it was poorly recorded.

In the 17th century, one of the best herbal remedies was introduced into Europe; the use of the bark of the Cinchona tree which became a popular medicine to treat malaria. Its active ingredient quinine was isolated two centuries later (1820). In the 18th century, the foxglove plant was used by Withering for treating dropsy and the active ingredient (digoxin) is currently being used for treating various heart conditions. The first 'pure' drugs became available during the 19th century. The alkaloids quinine (2, 1820), morphine (3, 1806), and ephedrine (4, 1887) were extracted from plants and chemists later on were able to disclose the chemical synthesis of these important alkaloids. A considerable number of drugs appeared in the second half of the 20th century that can treat diverse diseases as a result of the advancement in pharmaceutical science and the establishment of pharmaceutical companies. Although both synthetic and natural product-based drugs are currently used in curing diseases, numerous drugs in clinical use today are based on plant-derived natural products or their analogues. (Chowdhury, 2008).

1.5 Traditional Medicine:

Traditional medicine (TM) describes a group of health care practices and products with a long history of use. It frequently refers to medical knowledge developed by indigenous cultures that incorporates plant, animal and mineral-based medicines, spiritual therapies and manual techniques designed to treat illness or maintain wellbeing. 2 TM tends to be practiced outside of allopathic medicine (also known as biomedicine, conventional or Western medicine), which is the dominant system of medicine in the developed world. In many cultures, TM functions as a comprehensive system of health care refined over hundreds or even thousands of years. Some of the best-known TM systems include traditional Indian (Ayurveda) medicine, traditional Chinese medicine (TCM), and traditional Arabic (Unani) 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 illnesses. (Queiroz, 2009).

1.6 Antioxidants:

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves.

Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid et al. 2010). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, muscular dystrophy, liver disorder, and even aging (Amit and Priyadarsini 2011). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet (Teresa et al. 2011).

1.7 Classification of Antioxidants:

Antioxidants can also act as pro-oxidants when these are not present at the right place at the right concentration at the right time (Tourino, 2008).

Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic antioxidants.

1.7.1 Natural Antioxidants:

Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their

physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and non-enzymatic antioxidants.

1.7.1.1 Enzymatic Antioxidants:

Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into primary and secondary antioxidant.

1.7.1.2 Primary Antioxidants:

Primary antioxidants mainly include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as described below:

Superoxide Dismutase Superoxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O .−) and repairs the body cells damaged by free radical. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide (6.1). SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, it promotes the activity of NO (Chakraborty, 2009).

1.7.2 Secondary Antioxidant:

Secondary antioxidant includes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is required to recycle the reduced glutathione (GSH) using secondary enzyme GR and NADPH.

GSSG + NADPH ¾G¾R ® NADP + 2GSH

Glutathione is a cysteine containing peptide-type antioxidant and is synthesized in the body cells. The thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. A high level of glutathione is found in the cells (~3,100 μg/g of tissue) (Hissin and Hilf 1976), maintained in the reduced form (GSH) by the enzyme GR, and in turn reduces other metabolites and enzyme systems, such as ascorbate. Due to its high concentration and its role in maintaining redox state in the cells, it is considered one of the most important cellular antioxidants.

1.7.2.1 Non enzymatic Antioxidants:

They are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism (Raygani et al. 2007). Some of the known non enzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants as listed below.

1.7.2.2 Minerals:

Minerals are required in the body cells for the proper functioning of the enzymes. Their absence is known to affect the metabolism of many macromolecules. They include selenium, copper, iron, zinc, and manganese. They act as cofactors for the enzymatic antioxidants.

1.7.2.3 Iron (Fe):

Iron is the most abundant trace metal found to bound with protein in the biological system. Normally the concentration of free iron is very low and the low concentrations of iron-binding proteins promote ROS production, lipid peroxidation, and oxidative stress (Dabbagh et al. 1984). Hence iron supplementation helps in reducing the oxidative stress.

1.7.2.4 Magnesium (Mg):

Magnesium is a cofactor for glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) involved in pentose cycle which catalyzes the production of NADPH from NADP during the glucose metabolism and hence maintains the normal ratio of GSH to GSSG and a normal redox state in cells. Deficiency of magnesium reduces GR activity and GSSG does not reduce to GSH, hence causing oxidative damage to the cells (Fang et al. 2002).

1.7.2.5 Selenium (Se):

Selenium is also a very important component of enzymatic antioxidant. In the presence of selenium (Se), glutathione peroxidase (GPx) plays a protective role against oxidation of lipid and protects the cell membrane and takes part in H2O2 and lipids' hydroxyperoxide metabolism. Hence, Se behaves like vitamin E and can be substituted in place of vitamin E and is used to prevent the risk of cancer and cardiovascular diseases (Sikora et al. 2008).

Copper (Cu), Zinc (Zn), and Manganese (Mn) SOD is a class of enzyme that consists of different types of SODs, depending upon their metal cofactor such as Cu–Zn and Mn. Cu–Zn SOD is found in the cytosol having Cu and Zn at their active sites which helps in proton conduction, whereas Mn-SOD is found in mitochondria and has Mn at its active site. These metals are responsible for SOD's antioxidant activities.

1.7.2.6 Vitamins

Vitamins form the class of micronutrients required for the proper functioning of the body's antioxidant enzyme system, such as vitamin A, vitamin C, vitamin E, and vitamin B. They cannot be synthesized in our body and hence need to be supplemented in the diet.

1.7.2.6.1 Vitamin A:

Vitamin A is helpful in night vision and in maintenance of epithelial cells in mucus membranes and skin. Because of its antioxidant properties, it assists immune system also and is found in three main forms: retinol, 3,4-didehydroretinol, and 3-hydroxyretinol. The main sources of this include sweet potatoes, carrots, milk, egg yolks, and mozzarella cheese.

1.7.2.6.2 Vitamin C :

Vitamin C is water soluble and is also called as ascorbic acid. It is found in fruits (mainly citrus), vegetables, cereals, beef, poultry, fish, etc. It is helpful in preventing some of the DNA damage caused by free radicals, which may contribute to the aging process and the development of diseases, such as cancer, heart disease, and arthritis.

1.7.2.6.3 Vitamin E:

Vitamin E is a lipid-soluble vitamin. This consists of eight different forms such as α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Most abundantly found in almonds, safflower oil, soybean oils, oil of wheat germs, nuts, broccoli, fish oil, etc., α-tocopherol possesses highest bioavailability and is the most important lipid-soluble antioxidant which reacts with the lipid radical and protects the membranes from lipid peroxidation; as a result, oxidized α-tocopheroxyl radicals are produced that can be recycled to the reduced form through reduction by other antioxidants, such as ascorbate and retinol.

1.7.2.7 Carotenoid:

Carotenoid consists of β-carotene, lycopene, lutein, and zeaxanthin. They are fat soluble colored compounds found in fruits and vegetables. β-Carotene is found mostly in radish-orange-green color food items including carrots, sweet potatoes, apricots, pumpkin, mangoes, and cantaloupe along with some green and leafy vegetables, including collard greens, spinach, and kale. Lutein is abundant in green leafy vegetables such as collard greens, spinach, and kale (Hamid et al. 2010). Lutein is best known for its role in protection of retina against harmful action of free radicals and also prevents atherosclerosis (Sikora et al. 2008).

Although lycopene, lutein, canthaxanthin, and zeaxanthin do not possess pro vitamin A activity, β-carotene is known as a precursor for vitamin A (Fang et al. 2002). Tomato is a good source of lycopene and spinach is a good source of zeaxanthin. It has been shown that lycopene is a potent antioxidant and is the most effective compound in removing singlet oxygen found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, and other foods.

1.7.2.8 Polyphenols:

Polyphenols is a class of the phytochemicals that possess marked antioxidant activities. Their antioxidant activities depend on their chemical and physical properties which in turn regulates the metabolism depending on their molecular structures (Ajila et al. 2011). These consist of phenolic acids, flavonoids, gingerol, curcumin, etc.

Flavonoid is a major class of polyphenolic compound and is mostly found in vegetables, fruits, grains, seeds, leaves, flower, bark, etc. Some of the spices, such as ginger and turmeric, are also good sources of polyphenolic compound, e.g., gingerol is obtained from the rhizomes of ginger, whereas curcumin (diferuloylmethane) is the main bioactive component of turmeric and is known to possess good antioxidant activity. Curcumin is an excellent scavenger of ROS, such as O2 radicals, lipid peroxyl radicals (LO2), OH radicals, and nitrogen dioxide (NO2) radicals, which induced oxidative stress. Curcumin has been shown to inhibit lipid peroxidation and has been shown to increase GSH levels also in epithelial cells which lead to lower ROS production (Biswas et al. 2005).

1.8 Other Antioxidants:

Transition Metal-Binding Proteins Albumin, ceruloplasmin, hepatoglobin, and transferrin are the transition metal-binding proteins found in human plasma, bind with transition metals, and control the production of metal catalyzed free radicals. Albumin and ceruloplasmin are the copper ion sequesters, hepatoglobin is hemoglobin sequester, and transferrin acts as free iron sequester.

Non protein Antioxidants Bilirubin, uric acids, and ubiquinol are nonprotein antioxidants which inhibit the oxidation processes by scavenging free radicals (Papas 1998).

1.8.1 Bilirubin:

Bilirubin is an end product of heme catabolism. It is a lipid-soluble cytotoxic product that needs to be excreted. However, bilirubin efficiently scavenges peroxyl radical at micromolar concentrations in in vitro model (Stocker et al. 1987) and is regarded as the best antioxidant against lipid peroxidation.

1.8.2 Uric Acid:

Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. Urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin and protects erythrocytes from peroxidative damage. The plasma-urate levels in humans are about 300 μM, making it one of the major antioxidants in humans.

1.8.3 Coenzyme Q:

Coenzyme Q is also known as ubiquinol (Co Q) and is an oil-soluble antioxidant. This is produced in the body through monovalent pathway, in heart, liver, kidney, pancreas, etc. The mechanism of the action may occur in two ways:

In the first mechanism, reduced form of ubiquinol (CoQH) acts as chain-breaking antioxidant and reduces peroxyl (ROO.) and alcoxyl radicals (LO.) (Papas 1998).

CoQH + ROO. ® Q. + ROOH

In the second mechanism, it reacts with vitamin E radical (TO.) and regenerating vitamin E.

CoQH + TO. ® Q. + ROOH

1.9 Characteristics of Medicinal Plants:

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

1.9.1 Synergic Medicine:

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

1.9.2 Support of Official Medicine:

In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

1.9.3 Preventive Medicine:

It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Bassam Abdul Rasool Hassan, 2012).

1.10 Importance of Medicinal Plants:

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in the history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible for curative action of the herbs. Before onset of the synthetic era, man was completely dependent on medicinal herbs for the prevention and treatment of diseases. With introduction of the scientific procedures the researchers were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs some of which are therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples.

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

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

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

Diabetes mellitus is another area where a lot of research is going on. Ajuga reptens (the active principle is said to potentiate effects of insulin), Galagea officinalis (galagine), Bougainvillea spectabilis (pinitol), Momordica charantia (chirantin), Gymnema sylvestre (gymnemic acid) are some medicinal herbs that have shown effectiveness in non-insulin dependent diabetes. Recently extract of Tecoma stans has shown potent anti-diabetic activity. Alkaloid tecomonine is considered to be active principle of the herb.

Arthritis is another potential disease where no satisfactory answer is present in modern medicine. Commiphora mukul (guggulsterones), Boswellia serrata [boswellic acid], Withania somnifera (withanolides), Ruscus acueleatus (ruscogenin), Harpagophytum procumbens (harpagoside) are prominent plants with anti- arthritic activity. Harpagoside is a precious constituent as it has anti rheumatoid activity. Rest of all natural products has anti-inflammatory activity.

Chrysanthemum parthenium traditionally known as feverfew has shown promising results in migraine, a disease that has eluded the researchers from centuries. The herb contains sesquiterpenes lactones called parthenolides, which are the active principles of the herb. Hepatoprotective action of certain botanicals deserves attention. Sedum sarmentosum [sarmentosin], Schisandra chinensis [waweizichun and schisantherin] have shown their ability to lower raised liver enzymes in viral hepatitis.

Croton sublyratus [plaunotol] has potent and wide spectrum anti peptic ulcer action. A number of plant derivatives have shown anti-Aids activity. Ancistrocladus korupensis [michellamine-b], Caulophyllum langigerum [calanolide-a], Caulophyllum teymani

[costatolide-a], Homalanthus nutans [prostratin], Conospermum sp [concurvone] are the medicinal herbs from African countries that are being employed in research for finding a suitable cure for Aids.

The concept of antioxidants is fastly catching up and latest research has shown that a number of herbal derivatives have excellent antioxidant action. Bacopa monnieri contains bacosides A and B and bacoside A is a strong antioxidant, which reduces several steps of free radical damage. Coleus forskohlii [forskolin], Grape seed [proanthocyanidins], Camellia sinensis [polyphenols], Huperzia serrata [huperzine], Pinus maritima [Pycnogenol], Borago officinalis [gamma linoleic acid] and Vinca minor [Vinpocetine] are potential antioxidants.

The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to phytochemical screening for the detection of various plant constituents.

With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60%of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases. (Singh, 2016).

1.11 Some Common Medicinal Plants in Bangladesh:

Table 1: Common Medicinal Plants in Bangladesh

Common Name	**Botanical Name**	**Source of Drug**	**Uses**
Nayantara	*Catharanthus roseus*	Leaves	Cancer, Insomnia, Blood Pressure, Diabetes
Shatamuli	*Asparagus racemosus*	Roots	Cancer, Diabetes, Jaundice

Sarpagandha	*Rauvolfia serpentain benth.*	Roots	Insomnia, Brain disorder, Dysentery
Ghritkumari	*Aloe indica*	Leaves	Constipation, Fistula, Piles, Burns, Jaundice
Lajjabati	*Mimosa pudica*	Whole plants	Blood purification, toothache, piles
Patherkuchi	*Kalanchoe pinnata*	Leaves	Cough, Diabetes, Lung infection, wounds
Pudina	*Menthe arventsis*	Whole plant	Indigestion, stomach disorder, stimulant
Shimul	*Bombax ceiba*	Roots, Leaves,Barks,Flowers,Fruits	Fever, leprosy, pox, diarrhea, dysentery
Basak	*Adhatoda vasica*	Roots, Leaves, Flowers	Cough,asthma,arthritis,malaria
Sharisa	*Brassica napus*	Leaves, seeds	Fever, common cold, stomachache, headache
Dhutara	*Datura metel*	Roots, leaves, seeds	Anesthesia, pain, asthma, epilepsy, hypertension
Nisinda	*Vitex negunda*	Leaves, Barks	Weakness, cough, headache, malaria
Helencha	*Enhydra fluctuans*	Whole plants	Nutrition, bronchitis, skin disease, blood purification
Amloki	*Phyllanthus emblica*	Barks, flowers, fruits	Tonic, cough, diuretic, stomachache, dermatitis

(Ghani, 1998)

1.12 Families of Medicinal Plants:

Most of the medicinal and aromatic plants belong to the following families:

A. Medicinal plants of the Compositae family The Compositae family, also known as the Daisy family, contains the highest number of medicinal plants as compared to other families. Medicinal plants belonging to this family include the chamomile, the field and pot marigolds, daisy, wormwood, chicory, thistles, ragwort and artichoke.

- Chamomile. *Matricaria chamomilla.*

- True chamomile, *Anthemis nobilis.*

- Marigold. *Calendula.*

- Daisy. *Bellis annua,*

- Wormwood. Also known as *Artemisia absinthium,*

- Chicory. *Cichorium intybus* and *Cichorium spinose*

- Thistles. Milk thistle, known as *Silybum marianum*.

- Silver ragwort. *Senecio bicolour.*

- Artichoke, *Cynara cardunculus.*

B. Medicinal plants of the Labiatae family A very important medicinal plant family is the Labiatae family, also known as the mint family. Plants in this family are herbs or shrubs often with an aromatic smell. They are common in the Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Some examples from this family include horehound, lavander, balm, micromeria, the mints, thyme and rosemary. The lavander is a term given to a group of plants that have similar shape and properties. In Spain there are several species and subspecies.

- *Lavandula officinalis.*

- *L. angustifolia* Miller

- *L. angustifolia* Miller subsp. *pyrenaica*

- *L. latifolia* Medicus

- *L. lanata* Boiss.

- *L. dentata* L.

-*L. stoechas* L.

- *L. pedunculata* Cav.

- *L. viridis* L'Hér.

- *L. multifida* L.

The mints constitute a large group of plants. Their scent varies from pungent to sweet. These properties are owned by the distinct mint species.

- Pennyroyal (*Mentha pulegium*).

- Water mint *(Mentha aquatica).*

- Peppermint *(Mentha piperita)*

- Spearmint *(Mentha spicata)*

Thyme has also many representatives is Europe.

- Mediterranean thyme (*Thymus capitatus)*

- *Thymus granatensis* (red thyme)

- *Thymus hyemalis* (sauce thyme)

- *Thymus longiflorus* (long flower thyme)

- *Thymus mastichina* (Mejorana)

- *Thymus serpylloides* (sierra thyme)

- *Thymus vulgaris* (common thyme)

- *Thymus zygis* (olive thyme)

Finally we can find rosemary *(Rosmarinus officinalis*), Horehound (*Marrubium vulgare*), Balm, also known as *Melissa officinalis*, *Micromeria* (*Micromeria microphylla*).

C. Medicinal plants of the Umbelliferae family The Umbelliferae or carrot family consists of plants with a characteristic umbrella-arranged fruit. These plants usually produce an essential oil, an asset to survive during the hot summer days. In fact the oil has a cooling effect on the plant. Some examples from this family include bullwort (*Ammi majus*), wild celery (*Apium graveolens*), wild carrot (*Daucus carota*), sea holly *(Eryngium maritima*), fennel (*Foeniculum vulgaris),* anise (*Pimpinella anisum*), wild parsley *(Petroselinium crispum*), hemlock *(Conium maculatum*) and alexanders (*Smyrnium olusatrum*).

- Bullwort (*Ammi majus*).

- Wild celery (*Apium graveolens*).

- The wild carrot (*Daucus carota*)

- The sea holly (*Eryngium maritimum*)

- Fennel (*Foeniculum vulgare*).

- Parsley or Petroselinum.

- One of the most poisonous herbs is the spotted hemlock or *Conium maculatum*.

- Alexanders (*Smyrnium olusatrum*).

D. Medicinal plants of the Leguminosae family The Leguminosae or pea family consists of large number of plants, both native and naturalised, that have been cultivated for fodder, food and ornamental purposes. Amongst these plants, those with medicinal virtues include the carob tree (*Ceratonia siliquia*), the pea *(Pisum sativum*), white and red clovers (*Trifolium repens* and *pratense*), false acacia (*Robinia pseudoacacia*), Judas tree (*Cercis siliquastrum*), alfalfa (*Medicago sativa*) and fenugreek (*Trigonella foenumgraecum*). A group of closely related species in the Leguminosea family are the clovers. Two important species are the white and red clovers (*Trifolium repens* and *T. pratense*).

E. Medicinal plants of the Rosaceae family A large of species in Rosaceae or rose family, have a medicinal value. Most of these are trees or shrubs with variable characteristics. This family is popular for its edible and juice fruit shrubs and trees. Some examples of this family include bramble (*Rubus ulmifolius*), rose (*Rosa gallica*), wood strawberry (*Fragaria moschata*), quince (*Cydonia oblongata*), round pear (*Pyrus amydaliformis*), loquat (*Eriobotrya japonica*), hawthorn (*Crataegus monogyna*), peach, almond and apricot (*Prunus persica, amygdalus* and *armeniaca*).

- Bramble (*Rubus ulmifolius*)

- The wild rose (*Rosa gallica*)

- Wood strawberry (*Fragaria moschata*)

- Quince (*Cydonia oblongata*)

- The round pear (*Pyrus amydaliformis*).

- Hawthorn (*Crataegus monogyna*)

The Prunus genus includes several stone fruits such as the peach, almond, apricot, plum and blackthorn. Most of these are cultivated for their fruit, as all have a local market. However, most have naturalised in valleys and gardens.

- Peach *(Prunus persica*)

- Almond (*Prunus dulcis*)

- Blackthorn (*Prunus spinosa*)

F. Medicinal plants of the Rutaceae and Solanaceae families The Rutaceae or rue family is a small family that consists of cultivated fruit trees and medicinal herbs. Plants in this family include the wall and garden rues (*Ruta chalepensis* and *graveolens*), orange (*Citrus aurantium*), lemon (*Citrus limon*), tangerine (*Citrus paradisi*) and grapefruit (*Citrus paradisi*). The rues (*Ruta graveolens* and *R. montana*) are two related species that have different medicinal uses. A citrus tree with great medicinal value is the bitter orange tree (*Citrus aurantium*). A family with several poisonous, but medicinally-important herbs is the Solanaceae or potato family. A species in this family that is widely cultivated (*Solanum tuberosum*). Other cultivated edible crops are the tomato (*Lycopersicum esculentum*) and the aubergine (*Solanum melongena*). The potato is only edible when ripe, as green potatoes were found to be poisonous. Also although these three crops come from this poisonous family, through cultivation and experimentation, the genetic material that codes for the toxic compounds has been phased out, resulting in safer and non-toxic cultivars. Mediterranean natives in this family include the white henbane (*Hyoscyamus albus*), the Mediterranean withania (*Withania somnifera*) and garden thorn apple *(Datura metel*). Other important species include glaucous tobacco (*Nicotiana glauca*) and black nightshade (*Solanum nigrum).*

White henbane (*Hyoscyamus albus*).

- Mediterranean withania (*Withania somnifera*).

- Garden thorn apple (*Datura metel*).

- Stramonium *(Datura stramonium)*

- Glaucous tobacco *(Nicotiana glauca)*

- Black nightshade *(Solanum nigrum).*

G. Medicinal plants of the Cruciferae family The Cruciferae or cress family is characterised by plant that have flowers with cross-like petals. This family groups a large group of medicinal plants that include Wallflower *(Cheiranthus cheiri*), Bitter cress (*Cardamine hirsuta*), Shepherd's purse *(Capsella bursa-pastoris*), Black mustard *(Brassica nigra*), Horseradish (*Armoracia rusticana),* Hedge mustard (*Sisymbrium officinale*), White mustard *(Sinapis alba*), Wild radish *(Raphanus raphanistrum*), Watercress (*Nasturtium officinale).*

- Wallflower *(Cheiranthus cheiri*).

- Bitter cress *(Cardamine hirsuta*).

- Shepherd's purse (*Capsella bursa-pastoris*).

Although the Brassica plants are important crop, some of them have a medicinal value, such as the black mustard (*Brassica nigra*).

Other vegetable crops include cabbages and cauliflower.

- Horseradish *(Armoracia rusticana*).

- Hedge mustard (*Sisymbrium officinale*).

- White mustard (*Sinapis alba*).

- Watercress (*Nasturtium officinale*).

- Wild radish *(Raphanus raphanistrum*).

H. Medicinal plants of the Liliaceae family The Liliaceae or lily family is composed of large number of plant with medicinal virtues. Most of these are herbs and rarely shrubs. Examples from this plant family include Asphodel (*Asphodelus aestivus),* Wild asparagus (*Asparagus aphyllus*), Seaside squill *(Drimia maritima*), Mediterranean smilax *(Smilax aspera*), Greater butcher's broom (*Ruscus hypophyllum*), Butcher's broom *(Ruscus aculeatus*), Tassel hyacinth (*Muscari comosum*), Madonna lily *(Lilium candidum*), Bluebell (*Hyacinthus orientalis*), Aloe (*Aloe vera),* Garlic (*Allium sativum*), Garden onion (*Allium cepa*), Mediterranean meadow saffron (*Colchium cupani*), Meadow saffron *(Colchium autunnale)*

- Asphodel (*Asphodelus aestivus*).

- Wild asparagus *(Asparagus aphyllus*).

- Seaside squill (*Drimia maritima*).

- Mediterranean smilax (*Smilax aspera).*

Two closely related species are Butcher's broom (*Ruscus aculeatus*) and greater butcher's broom (*Ruscus hypophyllum).*

- Tassel hyacinth (*Muscari comosum*).

- Madonna lily (*Lilium candidum*).

- Bluebell (*Hyacinthus orientalis*).

- Aloe *(Aloe vera*).

- Garlic *(Allium sativum*).

- Garden onion *(Allium cepa*).

- Mediterranean meadow saffron (*Colchium cupani*).

- Meadow saffron (*Colchium autunnale*).

I. Medicinal plants of the Caryophyllaceae and Boraginaceae families The Caryophyllaceae or pink family group plants that usually have four to five petalled flowers that are usually white or pink in colour. Examples from this family include sandwort *(Arenaria serpyllifolia*), common chickweed (*Stellaria media),* sand spurrey (*Spergularia rubra*), nail wort (*Paronychia argentea*), smooth rupture-wort (*Herniaria glabra*), viscid sandwort (*Alsine tenuifolia*).

- Sandwort (*Arenaria serpyllifolia*).

- Common chickweed *(Stellaria media).*

- Sand spurrey (*Spergularia rubra*).

- Nail wort *(Paronychia argentea*).

- Smooth rupturewort (*Herniaria glabra*).

- Viscid sandwort *(Alsine tenuifolia*).

The Boraginaceae or borage family is made up of herbs or small shrubs with bristly stems and leaves. Examples in this family include borage *(Borago officinalis*), common comfrey (*Symphytum officinale*), purple alkanet (*Anchusa asurea*), yellow gromwell *(Neatostema apulum*), viper's bugloss *(Echium vulgare)* and southern hound's tongue (*Cynoglosum creticum*).

- Borage (*Borago officinalis*).

- Common comfrey (*Symphytum officinale*).

- Purple alkanet (*Anchusa azurea*).

- Yellow gromwell *(Neatostema apulum*).

- Viper's bugloss *(Echium vulgare*).

- Southern hound's tongue (*Cynoglosum creticum*).

J. Medicinal plants of the Ranunculaceae and Papaveraceae families The Ranunculaceae or buttercup family is characterised by showy flowers that usually have 5 petals. Examples from this family include pheasant's eye *(Adonis annuus*), lesser celandine *(Ranunculus ficaria*), poppy anemone (*Anemone coronaria*), love in the mist (*Nigella damascena*), short-spurred larkspur *(Delphinium staphysagria*), larkspur (*Delphinium ajacis*), traveller's joy *(Clematis vitalba),* evergreen traveller's joy *(Clematis cirrhosa*).

- Pheasant's eye *(Adonis annua*).

- Lesser celandine *(Ranunculus ficaria*).

- Poppy anemone (*Anemone coronaria).*

- Love in the mist (*Nigella damascena*).

- Larkspur *(Delphinium ajacis*).

- Short-spurred larkspur *(Delphinium staphysagria*).

- Evergreen traveller's joy *(Clematis cirrhosa*).

The Papaveraceae or poppy family consists of a group of plant that contain a latex or water sap. There are four petals in a flower and these are cross shaped with two opposite petals above the other two. Plants with a medicinal value include greater celandine *(Chelidonium majus*), opium poppy *(Papaver somniferum*), common poppy *(Papaver rhoeas*), sea poppy (*Glaucium flavum*), fumitory *(Fumaria officinalis*) and fumitory *(Fumaria capria capreolata*)

- Greater celandine *(Chelidonium majus).*

- Opium poppy *(Papaver somniferum).*

- The common poppy *(Papaver rhoeas).*

- The sea poppy *(Glaucium flavum*).

- Fumaria officinalis and Fumaria capria capreolata. The Latin name Fumaria means smoke of the earth, as these have a unpleasant smoky smell.

K. Medicinal plants of the Malvaceae and other families The Malvaceae or mallow family groups those plants that have five-petalled flowers and a nutlet-like fruit. Examples include common mallow (*Malva sylvestris*) hairless cotton *(Gossypium herbaceum*), hollyhock (*Althaea rosea*) and marsh mallow *(Althaea officinalis*).

- Common mallow, also known as *Malva sylvestris.*

- Hairless cotton (*Gossypium herbaceum*).

- Hollyhock (*Alcea rosea*).

- Marsh mallow (*Althaea officinalis*).

Other families that contain a very small number of medicinal plants include the following. The Cucurbitaceae or cucumber family contains a large number of edible crops such as the cucumbers, melons and pumpkins. Two important medicinal plants in this family include the squirting cucumber (*Ecballium elaterium*) and the pumpkin (*Cucurbita maxima*). Another family, called the Verbenaceae or verbena family contains three important medicinal plants; vervain *(Verbena officinalis*), chaste tree (*Vitex agnus-castus*) and the cultivated lantana *(Lantana camara*) An important and common medicinal plant of the Scrophularia or figwort family is the snapdragon *(Antirrhinum majus*). It is a native of West Mediterranean and grows on rocky grounds and old walls. It flowers from January till October. Traditionally it

was used as an astringent, diuretic, and hearmorrhoids. It contains several constituents such as alkaloids, amino acids and glycosides. A characteristic plant of the pokeweed or Phytolaccaceae family is pokeweed itself (*Phytolacca americana*). Due to its poisonous properties, it was used externally only, for the treatment of rheumatism, with low and skin inflammation. These are probably attributed to the saponins and the oleanolic acid derivative present in the plant. It contains a pokeweed lectin stimulates the white blood cells. A member of the Euphorbiaceae family castor bean (*Ricinus communis*) is renowned for its medicinal and industrial purposes. It is a native of the tropics but has naturalized in some waste places and valleys. IT flowers between March and October. Poisoning from seed ingestion has occurred in children. Traditionally, this plant was used as a laxative and to treat cradle-cap in babies. Castor oil is expressed from the seeds of the plant after they are peeled. Toxic albumins are present in the seed but these are removed by boiling with water. It is safe to use as a laxative and in baby skin products such as zinc and castor oil. It is used in industry as a lubricant to machinery and also in jet engines. (Gupta, 2008).

1.13 Review on Mangosteen (*Garcinia cowa*):

The mangosteen tree is very slow-growing, erect, with a pyramidal crown; attains 20 to 82 feet (6-25 m) in height, has dark-brown or nearly black, flaking bark, the inner bark containing much yellow, gummy, bitter latex. The evergreen, opposite, short-stalked leaves are ovate-oblong or elliptic, leathery and thick, dark-green, slightly glossy above, yellowish-green and dull beneath; 3 1/2 to 10 in (9-25 cm) long, 1 3/4 to 4 in (4.5-10 cm) wide, with conspicuous, pale midrib. New leaves are rosy. Flowers, 1 1/2 to 2 in (4-5 cm) wide and fleshy, may be male or hermaphrodite on the same tree. The former are in clusters of 3-9 at the branch tips; there are 4 sepals and 4 ovate, thick, fleshy petals, green with red spots on the outside, yellowish-red inside, and many stamens though the aborted anthers bear no pollen. The hermaphrodite are borne singly or in pairs at the tips of young branchlets; their petals may be yellowish-green edged with red or mostly red, and are quickly shed.

The fruit, capped by the prominent calyx at the stem end and with 4 to 8 triangular, flat remnants of the stigma in a rosette at the apex, is round, dark-purple to red-purple and smooth externally; 1 1/3 to 3 in (3.4-7.5 cm) in diameter. The rind is 1/4 to 3/8 in (6-10 mm) thick, red in cross-section, purplish-white on the inside. It contains bitter yellow latex and a purple, staining juice. There are 4 to 8 triangular segments of snow-white, juicy, soft flesh (actually the arils of the seeds). The fruit may be seedless or have 1 to 5 fully developed seeds, ovoid-

oblong, somewhat flattened, 1 in (2.5 cm) long and 5/8 in (1.6 cm) wide, that cling to the flesh. The flesh is slightly acid and mild to distinctly acid in flavor and is acclaimed as exquisitely luscious and delicious. (Borsani, C.,et al, 2004).

1.14 General Information:

Family: Clusiaceae

Bengali/vernacular name: Kau, Cowa, Kaglichu; Kao-gola (Chittagong)

Tribal name: Kao-gula (Chakma, Tanchangya), Tah Gala (Marma)

English name: Cow Tree

1.15 Taxonomical Classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Clusiaceae

Genus: *Garcinia*

Species: *Garcinia cowa*

1.16 Distribution:

Forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet.

1.17 Parts Used:

Leaves, barks and fruits.

1.18 General Description:

A medium-sized evergreen tree with horizontal branches and oval crown. Leaves 7.6-12.6 cm long, broadly to elliptically lanceolate, acuminate. Flower rather small, yellow; the male ones smaller in dense terminal clusters; the females 13 mm diam., or somewhat larger,

solitary or by 3-5 at the end of the branchlets. Berry the size of a lime, slightly 6-8 lobed, dul red, somewhat depressed at the apex.

Bark is astringent; used in spasm. Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery. Gum resin is drastic cathartic, may produce nausea and vomiting (Yusuf *et al.* 2009).

Ethanolic extract of the leaf possesses antibacterial properties (Anwar *et al*., 2007).

Fruit pericarp is composed of a fat and the seeds yield a wax-like fat consisting of glycerides of stearic, oleic, palmitic, linoleic and myristic acids. Bark contains a gum resin (Ghani, 2003). A new compound 1,3,6-Trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)-xanthone has been isolated from stems (Rastogi & Mehrotra, 1993).

1.19 Mode of Uses:

- Ripe fruits are edible, sour in taste, uncomfortable feeling in the mouth due to stick juice (Chakma).
- Ripe fruits are eaten, sour in taste (Khumi).
- Fruit is eaten when the dog is beaten by snake; the affected dog placed in a piece of leaves and also covered with leaves as the treatment (Murang).
- Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery (Tripura)
- Mangosteen twigs are used as chew sticks in Ghana. The fruit rind contains 7 to 14% catechin tannin and rosin, and is used for tanning leather in China. It also yields a black dye.
- **Wood:**

  In Thailand, all non-bearing trees are felled, so the wood is available but usually only in small dimensions. It is dark-brown, heavy, almost sinks in water, and is moderately durable. It has been used to make handles for spears, also rice pounders, and is employed in construction and cabinetwork.
- **Medicinal Uses:**

  Dried fruits are shipped from Singapore to Calcutta and to China for medicinal use. The sliced and dried rind is powdered and administered to overcome dysentery. Made

into an ointment, it is applied on eczema and other skin disorders. The rind decoction is taken to relieve diarrhea and cystitis, gonorrhea and gleet and is applied externally as an astringent lotion. A portion of the rind is steeped in water overnight and the infusion given as a remedy for chronic diarrhea in adults and children. Filipinos employ a decoction of the leaves and bark as a febrifuge and to treat thrush, diarrhea, dysentery and urinary disorders. In Malaya, an infusion of the leaves, combined with unripe banana and a little benzoin is applied to the wound of circumcision. A root decoction is taken to regulate menstruation. A bark extract called "amibiasine", has been marketed for the treatment of amoebic dysentery.

- The rind of partially ripe fruits yields a polyhydroxy-xanthone derivative termed mangostin, also ß-mangostin. That of fully ripe fruits contains the xanthones, gartanin, 8-disoxygartanin, and normangostin. A derivative of mangosteen, mangosin-e, 6-di-*O*-glucoside, is a central nervous system depressant and causes a rise in blood pressure.

1.20 Salient Feature of Family Clusiaceae:

Clusiaceae**,** the Garcinia family, in the order Malpighiales, comprising about 40 genera of tropical trees and shrubs. Several are important for their fruits, resins, or timbers.

Members of the Clusiaceae family usually have broad-ended, oblong leaves; these may be leathery and have a strong, central vein from which branch many delicate, horizontal veins. The plants have resinous, sticky sap, flowers with numerous stamens often united in bundles, and separate petals and sepals. Male and female organs often occur in separate flowers.

Scotch attorney, or cupey (*Clusia rose*), which is native to the Caribbean area, grows to about 10 metres (30 feet). It has leaves 10 cm (4 inches) long, flatly open flowers with six waxy, rosy-white petals, and many-seeded, multicelled, golfball-sized fruits. Like other species in the family, the fruits open and the valves spread widely like a star, exposing the succulent bright-orange tissue (arils) surrounding the seeds. Scotch attorney is planted as a beach shrub in areas exposed to salt spray.

Figure 1: Whole Plant of *Garcinia cowa*

C. grandiflora, which is native to Suriname, has larger flowers and ivory-white central stamen masses. Many members of the genus *Clusia* begin as epiphytes, or air plants, and eventually send roots over the host tree to the ground. All of the 300 to 400 members of the genus are tropical American.Mammee Apple or mamey (*Mammea americana*), native to tropical America, produces a grapefruit-sized, rough, russet-skinned, edible fruit. The other members of the genus *Mammea* are tropical but especially common in Madagascar.

Several trees of the genus *Garcinia* produce valuable fruits, such as the mangosteen (*G. mangostana*). Waika plum (*G. intermedia*), native to Central America, has a small, oval yellow fruit. There are 240 species in the tropics, being especially common in Indo-Malesia. Other members of the family, including beauty leaf (*Calophyllum inophyllum*) and Ceylon ironwood (*Mesua ferrea*), are cultivated as ornamentals in tropical regions. (Ghani 1998).

1.21 Chemical Constituents and Biological Activities of *Garcinia cowa* :

Many pharmaceutical drug discoveries originated from traditional folk medicine and its associated plant materials and bioactive secondary metabolites. The Genus Garcinia, belonging to the Family Clusiaceae which comprises about 300 species, have been widely investigated in terms of their bioactive ingredients. Native to Asia, Africa, South America and Polynesia, the plants are small to medium sized evergreen trees which may grow up to 30

m in height and are widely distributed in the tropical and temperate regions of the world. Twenty-nine species have been observed in Thailand, with 20, 13, 12, 7, 6 and 3 species found in the south, middle, north, east, north-east and west of the country respectively. Garcinia is a rich source of secondary metabolites, especially triterpenes, flavonoids, xanthones and phloroglucinols. The latter two groups are well recognized as cheomotaxonomic markers for this genus . Many of the isolated compounds have a wide range of pharmacological activities including anticancer, anti-inflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant and antioxidant.

Garcinia cowa, commonly known as Cha-muang in Thai, is widely distributed throughout Malaysia, Thailand and Myanmar. The fruits and young leaves are edible with a sour taste. The bark is dark brown with a yellow latex . The plant has unisex flowers: yellow orange female flowers found at the end of branches and male flowers found along the branches as clusters. The leaves are glossy, deep green, oblong and up to 6-15 cm in length and 2.5- 6.0 cm in width. The fruits are globose (2.5-6.0 cm in size), green when young and dull orange or yellow at maturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3-angled seeds. (Sharmin, T., et al, 2004)**.**

Figure 2: Flower of *Garcinia cowa*

Figure 3: Fruit of *Garcinia cowa*

Figure 4: Foliage of *Garcinia cowa*

Many parts of *G. cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an anti-fever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant. The chemical composition and biological activities of various parts of *G. cowa* have been investigated. The major compounds found were xanthones and phloroglucinols. However,

minor compounds, including depsidones, terpenoids, steroids and flavonoids, were also observed. Currently, 78 compounds have been isolated from the twig, stem, fruit and latex.

1.22 Distribution and Biological Activity:

The biological activities of the extracts from various parts of *G. cowa* have been investigated, including the hexane and chloroform extracts of the fruit rind and methanol extract of the leaves and twigs. The hexane and chloroform extracts from the fruit rind of *G. cowa* were tested against four Gram-positive bacteria (*Bacillus cereus*, *B. coagulans*, *B. subtilis* and *Staphylococcus aureus*) and one Gram-negative bacterium (*Escherichia coli*). Both extracts significantly inhibited bacterial growth of the Gram-positive bacteria (IC50s 15-30 μg/mL) but not *E. coli* (IC50s 250-500 μg/mL). The extracts were also found to inhibit the growth of *Aspergillus flavus* ATCC 46283, a common fungal food contaminant which produces aflatoxin B1. The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (ca 40-60% at the same concentration). The methanol extracts of the leaves and twigs of *G. cowa* were evaluated for their ability to inhibit low-density lipoprotein peroxidation induced by copper ions. The twig extract had an IC50 value of 20.5 μg/mL and was more potent (higher % inhibition at 1000 μg/mL) than the leaf extract (IC50 not measured). The twig extract was more potent than the leaf extract on platelet aggregation of human whole blood induced by arachidonic acid, adenosine diphosphate and collagen. These activities may be due to the total phenolic content of these extracts, which were 19 and 61 mg of gallic acid equivalent per gm of extract for the leaf and twig extracts respectively. (Schimmel, et al, 2004).

1.23 Classes of Compounds Isolated from *Garcinia cowa:*

1.23.1 Depsidone:

Depsidones comprise benzoic acid and phenol skeletons condensed at the ortho-positions through ester and ether linkages. This class of natural products is well known in the *Garcinia* species. However, cowadepsidone was the first and only known depsidone from *G. cowa*. It was isolated from the twig extract and showed cytotoxicity against NCI-H187 and MFC-7 cancer cell lines.

1.23.2 Flavonoids:

Twelve flavonoids were isolated from *G. cowa* with garccowasides A (6), B (7) and C (8) being first reported as new compounds. Of these compounds, only morelloflavone and morelloflavone-7′′-O-glucoside showed strong antioxidant activities. Phloroglucinols Phloroglucinols are based on a phloroglucinol or 1,3,5-benzenetriol core skeleton or its 1,3,5-cyclohexanetrione (phloroglucin) tautomer. The phloroglucinols found in *G. cowa* have a benzoyl group and geranyl and polyprenyl units as substituent groups. So far, fifteen phloroglucinols have been obtained from the twig including six new compounds: guttiferone K (15a), chamuangone (16), garcicowins A (17), B (18), C (21) and D (22), and nine known phloroglucinols: cambogin (14), guttiferones K (15b), B (25) and F(26), oblongifolins B (19), C (20), A (24) and D (27), and 30-epicambogin (23). Some of them showed selective cytotoxicity against two cancer cell lines (HT-29 and HCT-116) and normal colon cells (CCD-18Co). Guttiferone K (15) and 30-epicambogin (23) exhibited highest cytotoxicity against cancer cell line HT-29 [7]. The name Guttiferone K has been given to two different structures. Only one compound, chamuangone (16), was tested for its antibacterial activity and was found to be active against *S. pyogenes* (MIC = 7.8 µg/mL), *S. viridans* and *H. pylori* (MICs = 15.6 µg/mL), and *S. aureus*, *B. subtilis and Enterococcus sp*. (all of this bacteria shown MICs = 31.2 µg/mL) [27]. Terpenes and Steroids Terpenes and steroids represent two large classes of natural products, although they are rare in *G. cowa*. Only four of these types of compounds (5% of the total compounds isolated) were present in *G. cowa*, viz. friedelin, daucosterol, β-sitosterol and stigmasterol. None of these compounds were further studied for their biological activities. However, these compounds which were isolated from other plants had been investigated for their biological activities. Friedelin from the root bark of Terminalia avicennioides exhibited antibacterial activity against Bacillus Calmette-Guerin (BCG) with an MIC of 4.9 µg/mL. Friedelin and stigmasterol isolated from the leaf of Jatropha tanjorensis were tested against human pathogenic microorganisms, i.e. Gram-positive bacteria: *Bacillus cereus*, *B. subtilis*, *S. aureus* and *S. epidermis*; Gram-negative bacteria: *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *P. vulgaris*, *Salmonella paratyphi*, *S. paratyphi A*, *Vibrio alcaligenes* and *V. cholera*; and fungi: *Aspergillus fumigatus*, *Candida albicans*, *Microsporum gypseum* and *Trichophyton rubrum* using the agar-well diffusion and disk diffusion methods. Friedelin, at the concentration of 2 µg/mL, showed maximum activity with 37-40, 17-40 and 31-33 mm of clear zone diameter against these three types of

microorganisms respectively, while stigmasterol at the same concentration exhibited maximum activity with 13-15, 8-17 and 7-8 mm of clear zone diameter respectively. Daucosterol from the roots of *Astragalus membranaceus* had no growth-inhibitory effect by direct contact but possessed immunomodulatory effect against disseminated candidiasis caused by *Candida albicans*. β-Sitosterol and stigmasterol , isolated from the bark of *Grewia tiliaefolia*, at the same concentration of 1 µg/mL showed antibacterial activity against the Gram-negative bacterium *P. aeruginosa* (ATCC-20852) with 18 and 20 mm of clear zones respectively and against *Klebsiella pneumonia* (MTCC-618) with 15 and 15 mm of clear zones respectively as determined by the agar diffusion method.

1.23.3 Xanthones:

Xanthones, with two aromatic rings linked via carbonyl and ether linkages, are the major components of the Garcinia genus. They are commonly found in several parts of *G. cowa*, especially in the stem, fruit and latex. Thirty six xanthones (46% of the total isolated compounds) have been isolated and nineteen of them were first isolated from *G. cowa*. They are cowagarcinone, cowaxanthone, cowanol, cowanin, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl) xanthone, norcowanin, cowagarcinones A (49), B (50), E (51) and D (52) from the latex [15, 30]; cowaxanthones B (34), C (39), D (42) and E (44) from the fruit [20]; 7-O-methylgarcinone E (36), 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl) xanthone (59), 4-(1,1-dimethyl-prop-2-enyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl) xanthen-9 (9H)-one (61) and 1,5-dihydroxy-3methoxy-6′,6′-dimethyl-2H-pyrano (2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone (62) from the stem [18, 33]; and cowaxanthone F (55) from the twig. Most of these xanthones showed interesting biological activities.

1.24 Antibacterial Activity:

Eight xanthones from the fruit: cowaxanthones B (34) and C (39), 7-O-methylgarcinone E (36), α-mangostin, β-mangostin, mangostanin, cowanol and cowanin were investigated for their antibacterial activity against *S. aureus* and MRSA. α-Mangostin and mangostanin showed significant activity against these bacteria. α-Mangostin had a MIC value of 8 µg/mL against both *S. aureus* and MRSA while mangostanin had an MIC value of 4 µg/mL against both bacteria.

1.25 Anti-inflammatory Activity:

Eight xanthones: cowaxanthones A (32), B (34), C (39) and D (42), α-mangostin, mangostanin, cowanol and cowanin were tested for their anti-inflammatory activity using the ethyl phenylpropiolate induced ear edema assay. All xanthones except cowanol were more active than the standard drug, phenylbutazone. Antimalarial activity- Five xanthones isolated from the stem bark: 7-O-methylgarcinone, α-mangostin, cowaxanthone, cowanol and cowanin had significant in vitro antimalarial activity against *Plasmodium falciparum* with IC_{50} values ranging between 1.5-3.0 µg/mL. Anticancer activity Six xanthones: cowaxanthone, cowanol, cowanin, norcowanin, 3,6-di-Omethyl-γ-mangostin and dulxanthone isolated from twig were evaluated for their cytotoxicity against NCI-H187, KB, MFC-7 and/or HepG2 cell lines. Cowaxanthone, cowanin, norcowanin and 3,6-di-O-methyl-γ-mangostin exhibited significant cytotoxicity against the NCI-H187 cell line with IC_{50} values ranging between 3.87-8.58 µg/mL, and moderately inhibited KB and MCF-7 cancer cell lines with IC_{50} values ranging between 6.43-15.43 and 10.59-21.38 µg/mL respectively. Dulxanthone was found to be cytotoxic against the HepG2 cell line. (Academic library, 2014).

2.1 Literature Review on *Garcinia cowa:*

Though we have worked on leaf of this plant, we have studied on various parts of this plant. Here is some literature review on different parts of this plant.

2.1.1 Antioxidant and Antiplatelet Aggregation Properties of the Bark Extracts of *Garcinia pedunculata* and *Garcinia cowa*:

The bark extract of *Garcinia pedunculata* and *Garcinia cowa,* which is abundant in the Northeastern regions of India, were screened for their antioxidant and in vitro antiplatelet aggregating activities. By β-carotene linoleate model for antioxidant assay, acetone extract of *G. pedunculata* and hexane extracts of *G. cowa* exhibited higher antioxidant activity (86.47 and 66.94 % respectively, at 25 ppm) than other extracts. Similar pattern was observed for superoxide radical scavenging method for antioxidant assay. The ethyl acetate extract of *G. pedunculata* and hexane extract of *G. cowa* exhibited higher antiplatelet aggregation capacity towards ADP induced platelet aggregation (IC_{50} 0.16 and 0.43 ug, respectively) than other extracts. (Chowdhury *et al.*, 2013).

2.1.2 Cowaxanthone F and Other Anti-inflammatory and Antioxidant Compounds from *Garcinia cowa*:

A new tetraoxygenated xanthone, cowaxanthone F (1), as well as four known compounds, morelloflavone (2), volkensiflavone (3), morelloflavone-7 "-O-glucoside (fukugiside, 4), and 1,6-dihydroxyxanthone (5), were isolated from the crude acetone extract of the twigs of Garcinia cowa (Guttiferae). All compounds (1-5) were tested for antioxidant activity against DPPH (diphenylpicrylhydrazyl), hydroxyl, and superoxide radicals; only morelloflavone (2) and morelloflavone-7 "-O-glucoside(4) exhibited high potency. Eight tetraoxygenated xanthones from the fruits of G. cowa, cowaxanthones A-D (6-9), cowanin (15), alpha-mangostin (16), mangostanin (17), and cowanol (18), were also investigated for anti-inflammatory Activity using ethyl phenylpropiolate (EPP)-induced car edema. Assessment at 30, 60, and 120 min revealed that cowaxanthones B-D (7-9), cowanin (15), and alpha-mangostin (16) exhibited significant anti-inflammatory activity when compared to phenylbutazone, while cowaxanthone A (6), mangostanin (17), and cowanol (18) showed less activity. (Sharmin *et al.,* 2014)

2.1.3 Cytotoxic Compounds from the Leaves of *Garcinia Cowa:*

The aim of this study was to isolate compounds from the leaves of methanol extract of *Garcinia cowa* and to evaluated their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines. The dichloromethane fraction was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature these compounds were elucidated as methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate (1), garcinisidone-A (2) and methyl 4,6dihydroxy-2-(4-methoxy-5-(3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4-dienyloxy)-3-(3-methylbut-2-enyl)benzoate (3). Compound 1, 2 and 3 had IC50 value of 21.0 ± 10.2 µM, 21.2 ±8.4 µM and 17.2 ± 6.2µM against MCF-7, while only compound (2) was found to be in active against H-460 with IC50 value of 18.1 ± 6.7 µM. Conclusion: The results indicate that *G. cowa* leaves could be important sources of natural cytotoxic compounds and only compound (2) had activity against H-460 cell lines. (Zahed *et al.*, 2009)

2.1.4 Organic Acids from Leaves, Fruits and Rinds of *Garcinia Cowa:*

Organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa (G. cowa*) were determined by high-performance liquid chromatography. Fresh leaves, fruits, and dried rinds were extracted with water at 120 degrees C for 20-30 min under 15 lbs/in(2) pressure. Also, dried rinds were extracted with solvents (acetone and methanol) using a Soxhlet extractor at 60 degrees C for 8 h each. The samples were injected to HPLC under gradient elution with 0.01 M phosphoric acid and methanol with a flow rate of 0.7 mL/min using UV detection at 210 nm. The major organic acid was found to be (-)-hydroxycitric acid present in leaves, fruits, and rinds to the extent of 1.7, 2.3, and 12.7%, respectively. (-)-Hydroxycitric acid lactone, and oxalic and citric acids are present in leaves, fruits, and rinds in minor quantities. This is the first report on the composition of organic acids from *G. cowa*.

2.1.5 Updates on Antiobesity Effect of *Garcinia* Origin (-)-HCA (Hydroxycitric Acid):

Garcinia is a plant under the family of Clusiaceae that is commonly used as a flavoring agent. Various phytochemicals including flavonoids and organic acid have been identified in this plant. Among all types of organic acids, hydroxycitric acid or more specifically (−)-hydroxycitric acid has been identified as a potential supplement for weight management and as antiobesity agent. Various *in vivo* studies have contributed to the understanding of the anti-obesity effects of *Garcinia*/hydroxycitric acid via regulation of serotonin level and glucose

uptake. Besides, it also helps to enhance fat oxidation while reducing *de novo* lipogenesis. However, results from clinical studies showed both negative and positive anti-obesity effects of *Garcinia*/hydroxycitric acid. This review was prepared to summarize the update of chemical constituents, significance of *in vivo*/clinical anti-obesity effects, and the importance of the current market potential of *Garcinia*/hydroxycitric acid. (Okunji *et al*.,2016).

1.*Garcinia* has been used for centuries in Asian countries for culinary purposes as a condiment and flavoring agent in place of tamarind or lemon and to make meals more filling. Besides its use as a flavoring agent, the dried rind of *G. cambogia* combined with salt and other organic acids can help to lower the pH and thus provides a bacteriostatic effect in curing fish. *G. cambogia* contains large amounts of hydroxycitric acid (HCA). Similar to *G. cambogia*, *G. atroviridis* and *G. indica* also contain significant HCA content and are sometimes used interchangeably with *G. cambogia* in food preparation.

A myriad of health effects have been attributed to *Garcinia* (including *G. cambogia*, *G. atroviridis*, and *G. indica*), such as antiobesity effects, antiulcerogenic, antioxidative, antidiabetes, antimicrobial, antifungal, anti-inflammatory and anticancer effects. In particular, the anti-obesity effects of *Garcinia* or more specifically of its HCA content have been elucidated with unprecedented clarity over the last few decades. Besides its efficacy in the reduction of body weight and food intake, *Garcinia*/HCA has been proven to be beneficial in ameliorating obesity-related complications such as inflammation, oxidative stress, and insulin resistance. The results obtained from several studies supported the positive effects of HCA administration alone or in combination with other ingredients on body weight loss, reduced food intake, increased fat oxidation, or energy expenditure (EE) whereas some studies did not.

In spite of the vastly reported prominent role of HCA in inducing satiety, reduced energy intake and weight gain, and improved blood parameters and substrate oxidation, controversial results regarding its efficacy and safety as an anti-obesity dietary supplement had also been reported. Evidence from the *in vitro, in vivo*, and clinical trials on the safety of *Garcinia*/HCA as a dietary supplement for treating obesity had been extensively reviewed. However, the efficacy of *Garcinia*/HCA remains the subject of debate. Despite the previously stated issues, on conclusive evidence for HCA's efficacy in promoting weight loss and suppressing food intake, the marketing of a plethora of over-the-counter slimming aids containing HCA has taken place. The aim of this review is to critically assess the evidence

from a very broad range of reports, rigorous clinical trials, systematic reviews, and meta-analyses on the efficacy and potential of *Garcinia*/HCA as an anti-obesity dietary supplement.

2. *Garcinia* extract has been used in the traditional Ayurvedic medical system .A decoction of *G. cambogia* is given as purgative in the treatment of intestinal worms and other parasites, for bilious digestive conditions, for dysentery, rheumatism, and in the treatment of tumours. Less commonly, extracts are employed as cardiotonics to treat angina. In veterinary medicine, it is used as a rinse for diseases of the mouth in cattle. The fruit rind is used in rickets and enlargement of spleen and to heal bone fractures. In Southeast Asian folkloric medicine, a decoction of *G. atroviridis* (leaves and roots) is sometimes used for the treatment of cough, dandruff, earache, stomach pains associated with pregnancy, and throat irritation. The dried fruit of *G. atroviridis* is used for improving blood circulation, for the treatment of coughs, as a laxative, and as a expectorant. The fruit is used in a lotion with vinegar to rub over the abdomen of women after confinement. Fruit of *G. indica* is antiscorbutic, cholagogue, cooling, antibilious, emollient, and demulcent. The anthelmintic properties of the fruit of *G. indica* contributed to its use in haemorrhoids, dysentery, tumor, pains, and heart complaints. Bilious affected sites are treated with syrup from the fruit juice. Kokum butter is astringent and demulcent and is used in diarrhea and dysentery. It is also applied externally for ulcerations, sinuses, fissures of hand, lip, chapped skin, and skin diseases. (Willam *et al.*, 2012).

2.1.6 Cytotoxic and Nitric Acid Inhibitory Activities of Methanol Extracts of *Garcinia* Species:

The methanol extracts of 32 plant parts of 19 species of the genus *Garcinia* (Guttiferae) were collected from rainforests of the Malaysian Peninsula and the island of Sumatra, Indonesia, for evaluation of their *in vitro* cytotoxic and nitric oxide inhibitory activities. An end-point MTT cell viability assay was used to determine the 50% inhibitory concentration (IC_{50}) of the extracts in three human tumor cell lines representing tumors of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145). Griess assay was performed to assess the nitric oxide (NO) inhibitory activity. Of the 32 extracts, 27 showed cytotoxic activity in at least one of the three tumor cell lines used in this study. Four extracts, *Garcinia opaca* King (fruit), *Garcinia maingayi* Hook.f. (stem), *Garcinia penangiana* Pierre (leaf) and *Garcinia urophylla* Scortech. ex King (leaf) extracts showed the most potent and selective cytotoxic

activity against MCF-7 cells (IC_{50} 3-8 μg/mL). The extracts from *Garcinia cowa* Roxb. (stem), *Garcinia bancana* Miq. (stem) and *Garcinia malaccensis* Hook.f. (leaf) showed moderate activity and selectivity towards non-small lung tumor cells. The extracts from *Garcinia bancana* (stem), *Garcinia malaccensis* (stem), *Garcinia prainiana* King (leaf), *Garcinia rostrata* Hassk. ex Hook.f. (stem and leaf), *Garcinia cowa* (stem) and *Garcinia nervosa* Miq. (leaf) exhibited inhibition against NO production without affecting the viability of LPS and IFN-γ-induced RAW 264.7 macrophage cells. Among these, the most promising extracts were *G. bancana* (stem) and *G. malaccensis* (stem), as they showed the highest selectivity indices (> 50) for NO inhibition. In conclusion, these data provide evidence that some of the *Garcinia* species could potentially contain potent and selective cytotoxic and anti-inflammatory agents. (Gillbert *et al.,* 2012).

2.1.7 Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria:

The crude hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* were studied for their antibacterial activity against some foodborne pathogens and spoilage bacteria such as *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentrations (MICs) of the extracts determined by the agar dilution method were ranging from 15 to 500 μg/ml and 300 to 1250 μg/ml for *G. cowa* and *G. pedunculata*, respectively. However, the hexane and chloroform extracts from the fruit rinds of *G. cowa* exhibited marked inhibitory effect against all the test organisms and were more effective than that of *G. pedunculata* extracts. The antibacterial activity of all the extracts was more pronounced against the tested Gram-positive bacteria than the tested Gram-negative bacterium. Furthermore, this study is the first report on the *in vitro* antibacterial activity of extracts from the fruit rinds of *G. cowa* and *G. pedunculata*. (Sharmin, T., et al, 2004).

3.1 Theory of Phytochemical Screening:

3.1.1 Materials (Reagents and Tools) Used:

Table 2: Reagents Used in Phytochemical Screening

Reagents & Tools	
Molishch's reagents (10% naphthol in alcohol) - for carbohydrate test.	Conc. Hydroclric acid – for flavanoid test.
Dilute sulphuric acid and NaOH solution- for glycoside test.	Conc. Sulphuric acid- for steroid test.
Aqueous sodium hydroxide solution- for glycoside test.	$FeCl_3$ (5%) - for tannin test.
Fehling's solution- for glycoside test.	Solvents – alcohol, chloroform and distilled water.
10% Ammonia solution- for anthraquinone glycoside test.	Test tube
Mayer's reagent (potassiomercuric iodide solution)	Watch glass
Wagner's reagent (solution of I in KI)	Holder
Hager's reagent (Saturated solution of picric acid).	Burner
Dragendroff's reagent (Bismuth sub nitrate and acetic acid solution)- All for alkaloid tests.	

3.1.2 Test Compounds:

Dichloromethane extract of leaves of *Garcinia cowa*.

3.1.3 Preparation of Sample Solution:

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

3.1.4 Phytochemical Tests:

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

i. **Molisch's test for carbohydrates:** Two drops of molisch's reagents were added to about 5 mg of the extract in 5 ml aqueous solution in a test tube. 1 ml of conc. H_2SO_4 was allowed to flow down the side of the inclined test tube so that the acid formed a layer beneath the aqueous solution without mixing with in. a red ring was formed at the common surface of the two liquids which indicated the presence of carbohydrate. On standing or shaking a dark-purple solution was formed. Then the mixture was shaken and diluted with 5 ml of water. Dull violet precipitate was formed immediately.

ii. ***General test for glycosides*:** A small amount of extract was dissolved in 1ml of water then few drops of aqueous NaOH solution was added. A yellow color was developed in the presence of glycosides.

iii. ***Test for glycosides:*** A small amount of extract was dissolved in water and alcohol then boiled with Fehling's solution. Any brick-red precipitation was noted. Another portion of extract was dissolved in water and alcohol and boiled with a few drops of dilute H_2SO_4. The acid was neutralized with NaOH solution and boiled with Fehling's solution. A brick-red precipitation was produced in this experiment which showed the presence of glycosides in the extract.

iv. ***Borntragers's test for anthraquinone glycosides:*** 1 ml of sample solution was shaken with 5 ml of chloroform in a test tube for at least 5 minutes then again shaken with an equal volume of 10% ammonia solution. A bright pink, red or violet color was developed in the aqueous (upper) layer in the presence of free anthraquinones.

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

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

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

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

   d) ***Dragendroff's reagent:*** Formation of orange or orange-red precipitate indicated the presence of alkaloids.

vi. ***Test for saponins:*** about 0.5 ml of extract was shaken vigorously with water in a test tube. If a forthing was produced and it was stable for 1-2 minutes and persisted on warming, it was taken as preliminary evidence for the presence of saponins.

vii. ***Test for flavonoids:*** 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.

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

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

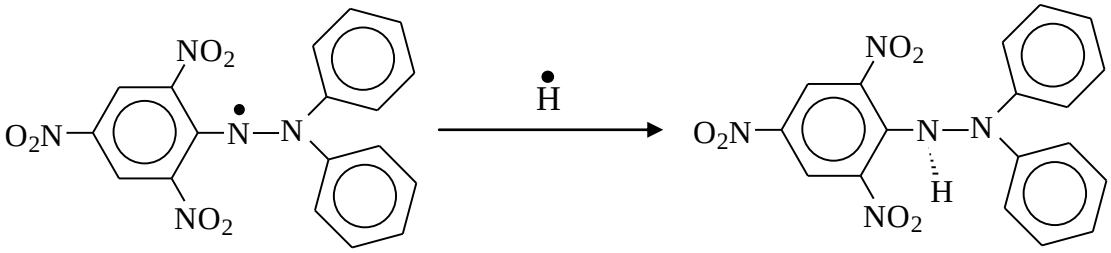
3.2 Assessment of In Vitro Pharmacological Property

3.2.1 Determination of Antioxidant property

3.2.2 DPPH Free Radical Scavenging Assay:

3.2.2.1 Principle:

DPPH is a reactive free radical that acts as an electron acceptor (oxidant/ oxidizing agent) and causes oxidation other substances. On the other hand, antioxidants act as electron donors (reductant/ reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as dark-colored crystalline powder composed of stable free-radical molecules and forms deep violet color in solution. The scavenging of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless.



1,.1-diphenyl-2-picrylhydrazyl **1,.1-diphenyl-2-picrylhydrazine**

Table 3: Reagents Used in DPPH Free Radical Scavenging Assay

Reagent	**Source**
Absolute Ethanol/Methanol	Merck, Germany
1,.1-diphenyl-2-picrylhydrazyl (DPPH	Sigma Chemicals, USA
Ascorbic acid (Analytical or Reagent grade)	SD Fine Chem. Ltd., Biosar, India

3.2.2.2 DPPH Solution:

0.004gm (4mg) DPPH is dissolved in 100 ml of solvent to make 0.004% solution.

3.2.2.3 Preparation of Standard/ Extract solution:

0.025 gm ascorbic acid or extract was taken and dissolved into 5 ml of Absolute ethanol. The concentration of the solution was 5mg/ml of ascorbic acid/extract. The experimental concentrations from the stock solution were prepared by the following manner:

Table 4: Amount of Standard or Extract Solution

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by Absolute ethanol	Final volume
800	320µl	-	1.68 ml	2.0 ml
400	-	1 ml(800µg/ml)	1 ml	2.0 ml
200	-	1 ml (400µg/ml)	1 ml	2.0 ml
100	-	1 ml (200µg/ml)	1 ml	2.0 ml
50	-	1 ml (100µg/ml)	1 ml	2.0 ml
25	-	1 ml (50µg/ml)	1 ml	1.0 ml
12.5	-	1 ml (25µg/ml)	1 ml	2.0 ml
6.25	-	1 ml (25µg/ml)	1 ml	2.0 ml

3.2.2.4 Procedure:

- The stock solution is serially diluted to achieve the concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml
- Each test tube contains 1ml of each concentration and is properly marked
- 2 ml of 0.004% DPPH solution in the solvent is added to each test tube to make the final volume 3 ml (caution: DPPH is light sensitive, so making the solution and adding it to the test tubes should be done in minimum light exposure)
- Incubate the mixture in room temperature for 30 minutes in a dark place
- Then the absorbance is measured at 517 nm against dilute extract solution in the solvent

3.2.2.5 Calculation:

$$\% \text{ Inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged/ neutralized and can be determined by linear regression method from plotting % inhibition against corresponding concentration.

3.3 Determination of Total Phenolic Content:

3.3.1 Principle:

The content of total phenolic compounds of plant extracts was determined as described previously (Velioglu*et al.,* 1998) using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton*et al.*, 1999).

However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds, Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}0_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

$Mo(VI) + e^{-} Mo(V)$

Table 5: Reagents Used in Determination of Total Phenolic Content

Reagent	**Source**
Folin - ciocalteu reagent	Merck, Germany E.
Sodium carbonate	Merck (India) Limited
Methanol	Merck, Germany
Gallic acid	Sigma Chemicals, USA

3.3.2 Preparation of 7.5% Sodium carbonate solution:

7.5 gm of Na_2CO_3 was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.3.3 Preparation of Standard solution:

The stock solution was prepared by taking 0.025 gm of galic acid and dissolved into 5 ml of Absolute Ethanol. The concentration of this solution was 5µg/µl of galic acid. The experimental concentrations from this stock solution were prepared by the following manner:

Table 6: Concentrations Used in the Preparation of Standard Solution

Concentration (µg/ml)	Solution taken from stock solution (µl)	Solution taken from others	Adjust the volume by distilled Ethanol (µl)	Final volume (ml)
200	80	-	1920	2
100	-	1 ml (200 µl/ml)	1000	2
50	-	1 ml (100 µl/ml)	1000	2
25	-	1 ml (50 µl/ml)	1000	2
12.5	-	1 ml (25 µl/ml)	1000	2
6.25	-	1 ml (12.5 µl/ml)		2

3.3.4 Preparation of Extract solution:

0.025 gm of each plant extracts were dissolved into 5 ml of Ethanol to make the concentration of each solution 5µg/µl of plant extract. These solutions were considered as stock solutions. The experimental concentration from these stock solutions was prepared by the following manner:

Table 7: Concentrations Used in the Preparation of Extract Solution

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled water (µl))	Final volume
200	40 µl	-	960	1.0 ml

3.3.4.1 Experimental Procedure:

1. 1.0 ml of plant extract (200µg/ml) or standard of different concentration solution was taken in a test tube.

2. 5 ml of Folin-Ciocalteu (Diluted 10 fold) reagent solution was added to the test tube.

3. 7.5% Sodium carbonate solution (4 ml) was added to the same test tube and mixed well.

4. Test tubes containing standard solutions were incubated for 30 minutes at 20°C to complete the reaction but the test tubes containing extract solution were incubated for 1 hour at 20°C to complete the reaction.

5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.

6. A typical blank solution contained the solvent used to dissolve the plant extract.

7. The Total content of phenolic compounds plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

C = (c x V)/m,

Where, C = total content of phenolic compounds, mg/gm plant extract, in GAE

c = the concentration of gallic acid established from the calibrationcurve (mg/ml)

V = the volume of extract in mlm = the weight of crude plant extract in gm

3.4 Antimicrobial Screening:

The antimicrobial activity of the plant extract was performed by the well accepted Bauer-Kirby method (Bauer *et al.,* 1966; Drew *et al.,* 1972).

3.4.1 Materials:

3.4.1.1 Microorganisms:

The microorganisms used in the antimicrobial activity assay of the extracts were carried out on both gram-positive and gram-negative bacteria.

3.4.1.2 Test Organisms:

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

Table 8: List of Test Bacteria

Gram positive Bacteria	**Gram negative Bacteria**
Bacillus cereus	*Escherichia coli*
Bacillus subtilis	*Salmonella typhi*
Staphylococcus aureus	*Pseudomonas aeruginosa*
	Serratiamarcescens
	Proteus mirrabillis

3.4.1.3Culture Media and Chemicals:

- Nutrient agar media
- Ethanol
- Chloroform

3.4.1.4 Equipments:

- Filter paper discs
- Petridishes
- Inoculating loop
- Sterile cotton
- Sterile forceps
- Spirit burner
- Micropipette
- Screw cap test tubes
- Nose-mask and Hand
- Laminar air flow hood
- Autoclave
- Incubator
- Refrigerator

3.4.1.5 Test Materials:

The dichloromethane extract of *Garcinia cowa* leaves were tested against gram-positive and gram-negative bacteria.

3.5 Methods:

3.5.1 Culture Preparation:

3.5.1.1 Composition of culture media:

Nutrient agar media with following composition is normally used to test the antimicrobial activity and to make subculture of the test organisms.

3.5.1.2 Composition of Nutrient agar media (1000 ml):

Table 9: Composition of Nutrient Agar Media

Ingredients	Amount
Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Sodium chloride	0.5 g
Distilled water	q.s. to 1000 ml
pH: 7.2 ± 0.1 at 250 C	

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 pH (at 25^0C) was adjusted at 7.2 ± 0.1 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 min. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study

3.5.1.3 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. Petridishes and other glasswares were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15 lbs/sq. inch for 20 min. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

3.5.1.4 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 h at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

3.5.1.5 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 suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media

3.6 Preparation of Discs:

3.6.1 Standard discs:

Standard discs 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 (10µg/disc) standard disc was used as the positive control.

3.6.2 Blank discs:

Blank discs 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 and did not influenced the results.

3.6.3 Preparation of sample discs with test samples:

20 & 30 mg of each test samples were dissolved in 1 ml of methanol to obtain the concentration 20µg/µl&30µg/µl 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 10 µl of solutions of test samples containing 400 µg and 800 µg of extract. Then the disks were dried.

3.6.4 Placement of Disc 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 40˚C for about 24 h. Finally the plates were kept in an incubator at 30˚C for 24 hr.

3.6.5 Determination of 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.

4.1 Phytochemical Screening of Dichloromethane Extract of *Garcinia cowa* Leaves

Table 10: Result of Phytochemical Screening of Dichloromethane Extract of *Garcinia cowa* Leaves

Carbohydrate	Alkaloid	Saponin	Alkaloid	Flavonoid	Glycoside	Tanin
-	-	-	+	+	-	+

4.2 DPPH Test of Dichloromethane Extract of *Garcinia cowa* Leaves

Table 11: Result of absorbance and % of inhibition of dichloromethane extract of *Garcinia cowa* leaves and ascorbic acid

Serial no.	**Concentration**	**Absorbance of sample**	**Absorbance of ascorbic acid**	**Percentage of inhibition of sample**	**Percentage of inhibition of ascorbic acid**
1	0	0	0	0	0
2	12.5	0.654	0.0986	23.953	70.663
3	25	0.326	0.0899	62.093	73.252
4	50	0.219	0.0647	74.535	80.749
5	100	0.161	0.0397	81.279	88.188
6	200	0.139	0.0245	83.837	92.710

4.2 Preparation of DPPH Scavenging Activity Curve:

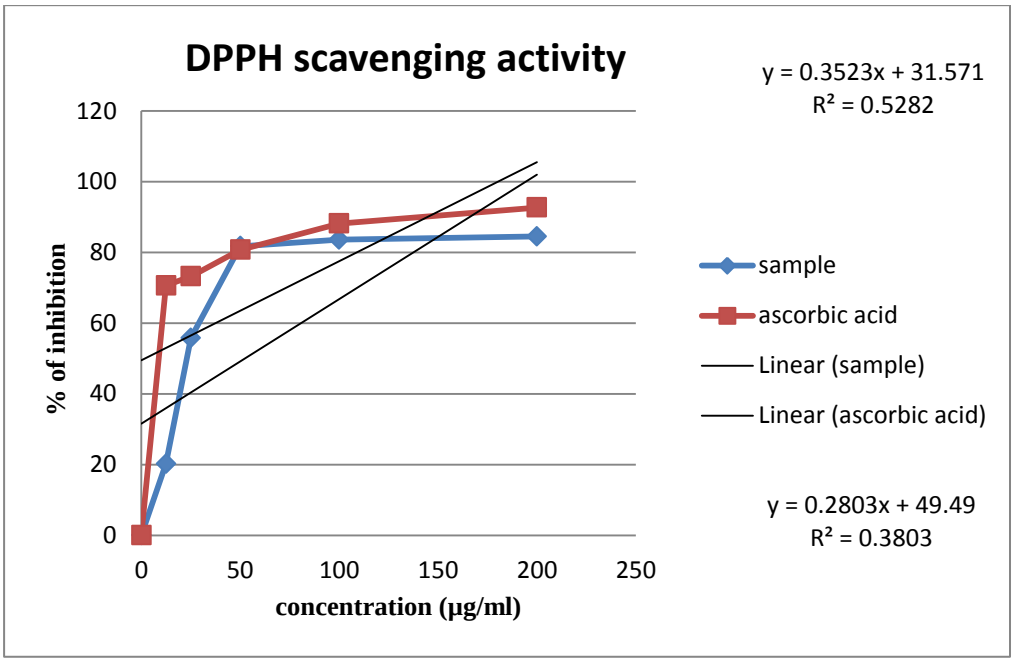


Figure 5: DPPH Scavenging Activity of Dichloromethane Extract of *Garcinia cowa* Leaves

4.2.1 Results of DPPH Test of Dichloromethane Extract of *G.cowa* Leaves

Table 12: Result of DPPH test of Dichloromethane Extract of *Garcinia cowa* Leaves

Dichloromethane Extract of G.cowa Leaves/ Ascorbic Acid	IC_{50} Value (µg/ml)	Regression Line	R^2Line
Dichloromethane Extract of *G.cowa* Leaves	51.7964	Y=0.334x+32.70	R^2= 0.530
Ascorbic Acid	1.8214	Y=0.280X+49.49	R^2= 0.380

4.3 Total Phenol Content of Dichloromethane Extract of *Garcinia cowa* Leaves

4.3.1 Preparation of Standard Curve for Gallic Acid:

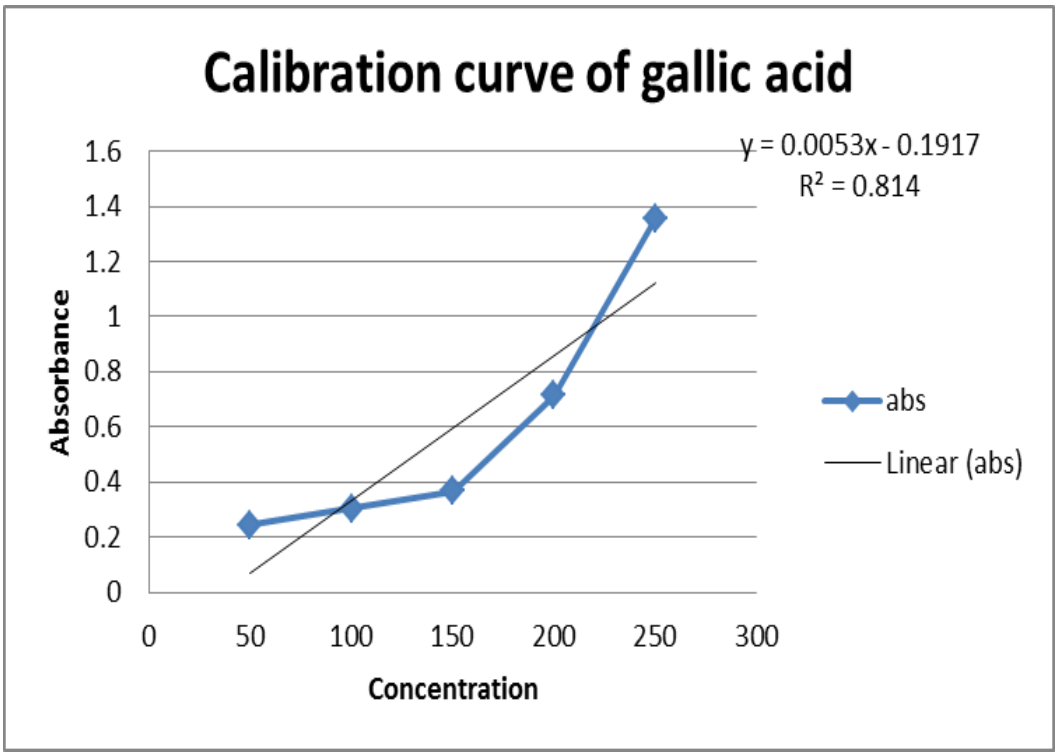


Figure 6: Standard Curve of Gallic Acid

4.3.2. Results of Total Phenol Content:

Table 13: Result of Total Phenol Content of Dichloromethane Extract of *Garcinia cowa* Leaves

Serial no.	Absorbance of Dichloromethane extract of *G.cowa* leaves	Standard equation	Value of x	Mean	Standard deviation	Total Phenol Content
1	0.673		172.8			
2	0.653	0.0053x-0.1917	168.8	173.93	5.78	173.93±5.78

3	0.71		180.2			

4.4. Antimicrobial Screening of Dichloromethane Extract of *Garcinia cowa* Leaves:

Table 14: Result of Zone of Inhibition of Dichloromethane Extract of *G.cowa* Leaves and Kanamycin

Name of Bacteria	Dichloromethane Extract of *G.cowa* leaves (400 µg/disc)	Dichloromethane Extract of *G.cowa* leaves (800 µg/disc)	Zone of Inhibition of Kanamycin (30µg/disc)
Bacillus sereus	7	8	25
Bacillus megaterium	-	-	25
Bacillus subtilis	-	8	28
Salmonella paratyphi	11	23	40
Salmonella typhi	-	9	26
Vibrio parahemolyticus	7	9	26
Staphylococcus aureus	-	-	25
E.coli	7	8	35
Shigella dysenteriae	-	-	25
Pseudomonas aureus	-	-	30

4.5 Discussion:

Herbal medicines have received high interest as a substitute to clinical treatment, and the demand for herbal remedies has currently increased rapidly. The increase in the number of herbal users as opposed to the insufficiency of scientific evidences on its safety has raised concerns regarding its detrimental effects and related concerns apply to the *Garcinia cowa* in this study.

Garcinia cowa is a medicinal plant enriched with various chemical constituents having different medicinal activities. The study has shown the antioxidant and antimicrobial activities.

It is used as a medicine for the treatment of various diseases. Dried fruits are shipped from Singapore to Calcutta and to China for medicinal use. The sliced and dried rind is powdered and administered to overcome dysentery. Made into an ointment, it is applied on eczema and other skin disorders. The rind decoction is taken to relieve diarrhea and cystitis, gonorrhea and gleet and is applied externally as an astringent lotion. A portion of the rind is steeped in water overnight and the infusion given as a remedy for chronic diarrhea in adults and children. Filipinos employ a decoction of the leaves and bark as a febrifuge and to treat thrush, diarrhea, dysentery and urinary disorders. In Malaya, an infusion of the leaves, combined with unripe banana and a little benzoin is applied to the wound of circumcision. A root decoction is taken to regulate menstruation. A bark extract called "amibiasine", has been marketed for the treatment of amoebic dysentery.

The aim of the present study is to evaluate the antioxidant activity and antimicrobial activity of Dichloromethane extract of *Garcinia cowa* leaves. Due to its huge therapeutic use by the tribal I get interested to do experiment on this plant. The therapeutic value of medicinal plants lies in the various chemical constituents in it.

Phytochemical screening showed that the dichloromethane extract of *Garcinia cowa* leaves was rich in phytochemical constituents. Such as- Flavonoid, Steroid, Carbohydrates and Tannin compounds. Thus further research is needed to work out the active medicinal compounds present in this extract; used for the treatment of various types of diseases.

In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect. In the present study the dichloromethane extract of *Garcinia*

cowa leaves showed the activity against *Bacillus sereus, Salmonella paratyphi, Vibrio parahemolyticus, E.coli* and plant based products have been effectively proven for their utilization as source for antimicrobial compounds.

The antioxidant activity was measured by Phytochemical Screening, DPPH and Total Phenol tests. IC_{50} values of DPPH tests were 51.7964 µg/ml for dichloromethane extract of *G.cowa* leaves. The Total Phenol contents 173.93±5.78 mg/g equivalent to Gallic Acid for Dichloromethane extract of *Garcinia cowa* leaves. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity.

It becomes difficult to describe the all properties selectively to any one group of constituents without further studies, which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active principles present in these plants.

The antimicrobial activity of the dichloromethane extract of *Garcinia cowa* leaves was tested against ten microorganisms. The highest antimicrobial activity was shown against *Salmonella paratyphi.* The diameter of the zone of inhibition was 11 mm (400µg/disc) compared to the 40 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc. It showed the moderate activity against *Bacillus sereus, Vibrio parahemolyticus* and *E.coli.* In case of 800µg/disc, the highest zone of inhibition of *Garcinia cowa* was 23 mm for *Salmonella paratyphi* where the zone of inhibition of Kanamycin was 40 mm. It showed no activity against *Bacillus megaterium, Staphylococcus aureus, Shigella dysenteriae* and *Pseudomonas aureus.* So the dichloromethane extract of *Garcinia cowa* leaves showed good antimicrobial activity against the selected microorganisms and thus further studies must be conducted to isolate the pure compounds and to evaluate their antimicrobial activity by using more advanced methods.

Conclusion

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. In my experiment it shows very positive result for anti-oxidant activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

References:

Academic library (2014). Biochemical assays for Antioxidant activity assessment. Available at: http://academlib.com/17949/environment/biochemical_assays_antioxidant_activity_assessment#665

Academic library (2014). Biotransformation of Waste Biomass into High Value Biochemicals. Available at: http://academlib.com/17945/environment/antioxidants.

Academic library (2014). Mechanism of Antioxidant Activity. Available at: http://academlib.com/17943/environment/mechanism_antioxidant_activity#668.

Andrea Rinaldi, Priya Shetty. (2010). Traditional medicine for modern times: Facts and figures. Available at: http://www.scidev.net/global/medicine/feature/traditional-medicine-modern-times-facts-figures.html.

Bassam Abdul Rasool Hassan (2012). Medicinal Plants (Importance and Uses). Available at: http://www.omicsonline.org/medicinal-plants-importance-and-uses-2153-2435.1000e139.php?aid=10875.

B.C. Bennett (n.d.) Twenty-five economically important plant families. Available at: http://www.eolss.net/sample-chapters/c09/e6-108-03.pdf.

Chowdhury S.R., Sharmin T., Hoque M., Sumsujjaman M., Das M. and Nahar F., (2013).

Evaluation of Thrombolytic and membrane stabilizing activities of four medicinal plants of Bangladesh. *International Journal of Pharmaceutical Science and Research,* Vol: 4(11), pp.4223-27.

Dr Xiaorui Zhang. (2000). Traditional Medicine: Definitions. Available at:

http://www.who.int/medicines/areas/traditional/definitions/en/.

eMonocot. (2010). *Garcinia cowa* Roxb. Available at: http://emonocot.org/taxon/urn:kew.org:wcs:taxon:304830.

Encyclopedia of Life (2013) Garcinia-Garcinia-classifications-encyclopedia of life. Available at: http://www.eol.org/pages/33634/names.

Evita Ochel. (2010). The Value and Importance of Plants in Medicine. Available at: http://www.evolvingwellness.com/post/the-value-and-importance-of-plants-in-medicine.

Jennifer Alinio. (2007). Importance of Medicine in Our Daily Lives. Available at: http://ezinearticles.com/?Importance-of-medicines-in-our-daily-lives&id=423789.

Kumaran A, Karunakaran AJ. (2007). In vitro antioxidant activities of dichloromethane extracts of five Garcinia species from India. LWT- Food Sci Technol; 40: 344-52.

My Agricultural Information Bank (2015). Classification of Medicinal Plants on the Basis of Parts Utilized. Available at: http://www.agriinfo.in/default.aspx?page=topic&superid=3&topicid=1409.

Pandey, R.P. & Dilwakar, P.G. (2008). An integrated check-list of flora of Andaman and Nicobar islands, India. Journal of Economic and Taxonomic Botany 32: 403-500.

Plantlife (2010). What are medicinal plants?. Available at: http://www.plantlife.org.uk/international/wild_plants/medicinal_plants_and_livelihoods/what_are_medicinal_plants/.

Rumana Huque (2014). The Use of Traditional Medicine: A study in Bangladesh. Available at: http://ghf.g2hp.net/2014/02/25/the-use-of-traditional-medicine-a-study-in-bangladesh/.

Sharmin, T., Sharmin, R., Chowdhury, M., Hoque, M., Sumsujjaman, M. and Nahar, F. (2014). Evaluation of antimicrobial activities of some Bangladeshi plants, *World Journal of Pharmaceutical Science 2014,* Vol: 2(2), pp: 170-175.

U.S. National Library of Medicine (2017). Antioxidants. Available at: http://medlineplus.gov/antioxidants.html.

Wordpress (2010). Importance of Medicinal Plants. Available at: https://ayuvedaherbs.wordpreess.com/.

Zahid (2016). Introduction and Importance of Medicinal Plants and Herbs. Available at: http://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl.