“Phytochemical Screening and Pharmacological Investigation of Petroleum Ether Extract of *Garcinia cowa* Stems”

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

AKM Saiful Alam

ID: 2013-3-70-007

Department of Pharmacy,

East West University.

East West University

DECLARATION BY THE CANDIDATE

I, AKM Saiful Alam hereby declare that the dissertation entitled “Phytochemical Screening and Pharmacological Investigation of Petroleum Ether Extract of *Garcinia cowa* Stems ” submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of Nazia Hoque, Assistant professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree or diploma or other similar title to any candidate of any university.

AKM Saiful Alam

ID: 2013-3-70-007

Department of Pharmacy,

East West University.

CERTIFICATE BY THE SUPERVISOR

This is to certify that the thesis entitled "Phytochemical Screening and Pharmacological Investigation of Petroleum Ether Extract of *Garcinia cowa* Stems " submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by **AKM Saiful Alam**, **ID: 2013-3-70-007** in 2017 of his research in the Department of Pharmacy, East West University, under my supervision and guidance.

_____

NAZIA HOQUE
Assistant professor.
Department of Pharmacy,
East West University.

ENDORSEMENT BY THE CHAIRPERSON

"Phytochemical Screening and Pharmacological Investigation of Petroleum Ether Extract of *Garcinia cowa* Stems " is an authentic research work done by **AKM Saiful Alam** , ID: 2013-3-70-007 under the guidance of **Nazia Hoque**, Assistant professor, Department of Pharmacy, East West University, Dhaka.

Dr. Chowdhury Faiz Hossain

Professor and Chairperson, Department of pharmacy,

East West University. Dhaka

Acknowledgement

At first, I would like to thanks the almighty “ALLAH” the most gracious and merciful for enabling me to successfully complete my research work soundly and orderly. I would like to express my sincere thanks to East West University for giving me the opportunity to conduct this study in the East West University laboratory of the Department of Pharmacy.

I feel proud to express my heartiest regards and deep sense of gratitude to my reverend teacher and supervisor **Nazia Hoque**, Assistant professor, Department of Pharmacy, East West University, for her day to day mastermind direction, constant supervision and support, constructive suggestions, optimistic counseling, valuable criticism, keen interest and active encouragement to carry out this research work.

It gives me immense pleasure to express sincere appreciation and gratefulness to **Dr. Chowdhury Faiz Hossain**, Chairperson, Department of Pharmacy, East West University for providing all possible help during the course of this research work. It is also great pleasure for me to offer my deepest indebtedness to all of my respected teachers of the Department of Pharmacy, East West University for extending their helping hands whenever needed.

I also would like to extend my thanks to the Lab officers of the Department of Pharmacy, East West University for their help and assistance whenever needed. I wish to thank my fellow researchers namely, Sabrina Islam, Farjana Sarwar Prova and Surovy Sharmin for their endless cooperation and whole hearted inspiration throughout the period of the research work which enabled me to work in a congenial and comfortable atmosphere.

Finally, I express my sincere thankfulness to my family members for guiding me all through my life, including that for my research project. I am especially grateful to my parents for their help, sacrifices, cooperation and inspirations in my work.

Dedication

This Research Paper is dedicated to

My beloved parents,

Who are my biggest inspirations…

Abstract

The aim of the present study was to evaluate phytochemical content, investigating the antioxidant and antimicrobial activity of petroleum ether extract of *Garcinia cowa* stem. The antioxidant activity was measured by DPPH and total Phenol test. *Garcinia cowa* 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. The IC_{50} values of DPPH scavenging activity was 246.875 µg/ml and the total Phenol content was 173.93±5.78 mg/g equivalent to Gallic Acid for Petroleum ether extract of *Garcinia cowa* stems. By determining antioxidant property, the result suggests that the plant extract possesses antioxidant property. The antimicrobial activities of petroleum ether extract of *Garcinia cowa* stems were tested against nine microorganisms by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The sample extract of *Garcinia cowa* stems showed good antimicrobial activities (zone of inhibition :11 mm-15 mm) against the microorganisms. The concentrations used in this study were 300 µg/ disc and 600 µg/ disc. Petroleum ether extract of *Garcinia cowa* stems showed highest activity against *Escherichia coli* and *Sarcina lutea* (zone of inhibition is 15mm) and mild to moderate activity against *Vibrio parahemolyticus, Shigella dysenteriae, Salmonella paratyphi* (zone of inhibition: 10-13 mm).

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.*

Keywords: *Garcinia cowa,* Clusiaceae, antimicrobial activity, antioxidant activity, zone of inhibition

CONTENTS

List of figures

List of Tables

Chapter 1: Introduction

1.1 General Introduction

Natural environment has been a source of medicinal agents for thousands of years, since healing with plants dates back probably to the evolution of *Homo sapiens*. Even to date, about 80% of the world's inhabitant's rely mainly on traditional medicines for their primary health care, while medicinal plants continue to play an important role in the health care systems of the remaining 20%. Partly based on their use in traditional medicine, an impressive number of modern drugs have also been isolated from natural plant species.

Most frequently, medicinal plants are defined as feral and/or cultivated plants that, based on tradition and literature records, can be directly or indirectly used for medical purposes. The basis for this use is that these plants contain active ingredients (active principles or biologically active principles) that affect physiological (metabolic) processes of living organisms, including human beings. The notion of aromatic plants is even less definite. The attribute aromatic indicates plants having an aroma; being fragrant or sweet-smelling, while the word aroma is supposed to imply also the taste of the material (aromatic herbs). Spice plants are plants used for seasoning, spicing, flavoring and coloring foods, drinks and different products of the food processing industry, i.e. making a product more enjoyable. (Sumner and Judith, 2000).

1.2 Definition of Medicinal Plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis". 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. (Ghani, 1998)

1.3 Importance of Medicinal Plant in Drug Discovery

Some synthetic chemicals may alter body's normal functioning if those are exogeneous. They may even cause mutation. Another problem is less structural diversity. Synthesis of complex structures may be time consuming and also is a costly process. Again, the selectivity of synthetic compounds may be less than the natural compounds as the natural compounds have more complex structures. Certain synthetic drugs can create addiction. In contrast, there are many natural drugs that are very important for use. Often synthetic drugs are made in their comparison but they lack in therapeutic effects than then the natural drugs.

As herbs are not without disadvantages, herbal medicine is not appropriate in all situations. For example, crude medicine can't be used in severe trauma, sudden illness and accidents. Moreover, the appropriate dose can't be determined. Herbs can be poisonous, especially when it is collected from wild sources. At the same time, chances of interaction are higher as no pure constituent is isolated.

So, the most appropriate approach would be chemical isolation of the lead compounds from the crude drugs and modification of the according to decrease the toxic risk. As the source would remain natural disadvantages of wholly synthetic agents could be overcome. Also, the problem of dose regimen and drug-interaction could be solved as the active principle would be isolated. (Galatea, 2016)

Medicinal plants play a very important role in the identification and synthesis of new chemical entities (NCEs). Between 1981 and 2002 approximately 28% of new entities were found from medicinal plants. Again 20% of synthetic compounds were also produced as mimicking agents of natural products. Natural products serve as the precursor for the synthesis of new synthetic compounds where the compounds have diverse structures and complex stereocenters, which

would be challenging to produce without the help of natural products. There are many structural features that are common to natural products (for example chiral centers, aromatic ring, degree of saturation) that are very relevant to drug discovery efforts.

Newer methods of drug discovery include combinatorial chemistry that also has the limitation of less diversity. To overcome this problem medicinal chemists are exploring with the creation of natural product libraries. These libraries combine the structural characteristics of natural products with new synthetic products that are generated by the combinatorial chemistry. With the help of medicinal plants drug optimization is also possible. If during drug discovery from medicinal plants, new chemical structure is not found then important drug lead is also possible with the help of the older compounds that may show new biological activities. Medicinal plants may show promising and selectivity in activity when high-throughput screening is done. Newly validated molecular targets are also being occupied by several compounds that are isolated from traditional medicinal plants.

Some examples of such isolates include indirubin and kamebakaurin that selectively inhibits cyclin dependent kinases and NFnB respectively. Not only these certain compounds are also useful to fight cancer. For example betulinic acid, an isolated compound from medicinal plant is useful against myeloma. (Balunas and Kinghorn, 2005)

1.4 History of Medicinal Plants

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

advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis. (Kelly et al, 2009)

1.4.1 Ancient Times

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

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

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

Gingko biloba

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

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

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Earliest Sanskrit writings such as the Rig Veda, and AtharvaVeda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system.(Sumner, Judith, 2000) Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources. (Dwivedi, 2007)

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

The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works

have survived intact, but from what remains, scholars have noted that there is a large amount of overlap with the Egyptian herbals. (Robson et al, 2009)

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

1.4.2 Middle Age

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

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

Avicenna's The Canon of Medicine lists 800 tested drugs, plants and minerals.Book was devoted to a discussion of the healing properties of herbs including nutmeg, senna, sandalwood, rhubarb, myrrh, cinnamon and rosewater. The Canon of Medicine remained a medical authority, used at many European and Arab medical schools, until the early 19th century. In particular, the Canon introduced clinical trials, randomized controlled trials and efficacy tests. (Huff and Toby, 2003)

1.4.3 Early Modern Era

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

1.5 Traditional Medicine

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

In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called alternative medicine. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional

Chinese medicine, traditional Korean medicine, acupuncture, and traditional African medicine.Core disciplines which study traditional medicine include herbalism, ethno medicine, ethnobotany, and medical anthropology. (WHO, 2015)

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

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

a) Folk medicine, which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting, bonesetting, hot and cold baths, therapeutic fasting and cauterisation.

b) Religious medicine, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods etc.

c) Spiritual medicine, which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

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

a) The Ayurvedic system which is the old Indian system

b) The Unani system which has been developed by the Arab and Muslim scholars from the ancient Greek system.Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. (WHO,2010)

1.5.1 Ayurveda

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

Originated in prehistoric times, some of the concepts of Ayurveda have been discovered since the times of Indus Valley Civilization and earlier. Ayurveda significantly developed during the Vedic period and later some of the non-Vedic systems such as Buddhism and Jainism also incorporated in the system. Balance is emphasized, and suppressing natural urges is considered unhealthy and claimed to lead to illness. Ayurveda names three elemental substances, the doshas (called Vata, Pitta and Kapha), and states that a balance of the doshas results in health, while imbalance results in disease. Ayurveda has eight canonical components, which are derived from classical Sanskrit literature. Some of the oldest known Ayurvedic texts include the Sushruta Samhita and Charaka Samhita, which are written in Sanskrit. (Maridass & Britto,2008)

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

1.5.2 Unani

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

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

Unani medicine first arrived in India around 12th or 13th century with establishment of Delhi Sultanate (1206–1527) and Islamic rule over North India and subsequently flourished under Mughal Empire. Alauddin Khilji had several eminent Unani physicians (Hakims) in his royal courts. In the coming years this royal patronage meant development of Unani practice in India,but also of Unani literature with the aid of Indian Ayurvedic physicians. (Arnold, 2000).

1.5.3 Traditional Chinese Medicine (TCM)

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

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

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

The doctrines of Chinese medicine are rooted in books such as the Yellow Emperor's Inner Canon and the Treatise on Cold Damage, as well as in cosmological notions such as yin-yang and the five phases. In the 1950s, the Chinese government promoted a systematized form of TCM. TCM's view of the body places little emphasis on anatomical structures, but is mainly concerned with the identification of functional entities (which regulate digestion, breathing, aging etc.). While health is perceived as harmonious interaction of these entities and the outside world, disease is interpreted as a disharmony in interaction. TCM diagnosis aims to trace symptoms to patterns of an underlying disharmony, by measuring the pulse, inspecting the tongue, skin, and eyes, and looking at the eating and sleeping habits of the person as well as many other things. (Shang et al, 2007)

1.6 Medicinal Plant: Center of Research

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

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

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

So, Plants have been the traditional source of raw materials for medicine. It is known through the scholastic works of AtharvaVeda and the writings of Charaka and Sushruta which gave huge knowledge of preventive and curative medicinal to the scientific community. Now, nearly 95% of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates

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

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

So, for being cheap, relatively safe and easily available, medicinal plants and herbs embody the foundation of traditional medicinal practice all over the world. Representing an untapped and huge reservoir of drugs either known or novel in origin, the medicinal plants are center of research to find out novel lead compounds. (Maridass et al. 2008).

1.7 Increasing popularity of medicinal plants

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

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

Low cost: herbals are relatively inexpensive and the cost of pharmaceuticals to governments and individuals is rising

Drug resistance: the need for alternative treatments for drug-resistant pathogens limitations of medicine: the existence of ailments without an effective pharmaceutical treatment.

Medicinal value: laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants.

Cultural exchange: expanding contact and growing respect for foreign cultures, including alternative systems of medicine.

Commercial value: growing appreciation of trade and other commercial economic opportunities represented by medicinal plants. However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine. (Duke 1993, Cox and Balick 1994).

1.8 Classification of Medicinal Plant:

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

Table 1.1 Based on the active constituents

Based on the active constituents:	
Aromatic Herbs	Fennel, Ginger, garlic, Lemon grass
Nerving Herbs	Ginger, Catnip
Astringent Herbs	Peppermint, Red raspberry

Bitter Herbs	Aloe, cascara, Liquorices
Mucilaginous Herbs	Althea, Aloe, Burdock, Comfrey
Nutritive Herbs	Acerola, Apple

Table 1.2 Based on plant part use

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

Table 1.3 Based on Therapeutic value

Based on Therapeutic value	
Antimalarial	*Cinchona officinalis, Artemisia annua*
Anticancer	*Catharanthus roseus, Taxus baccata*
Antiulcer	*Azadirachta indica, Glycyrrhiza glabra*
Antidiabetic	*Catharanthus roseus, Momordica charantia*
Anticholesterol	*Allium sativum*

Anti-inflammatory	*Curcuma domestica, Desmodium gangeticum*
Antiviral	*Acacia catechu*
Antibacterial	*Plumbago indica*
Antifungal	*Allium sativum*
Antiprotozoal	*Ailanthus* sp , *Cephaelisi pecacuanha*
Antidiarrhoeal	*Psidium gujava, Curcuma domestica*
Hypotensive	*Coleus forskohlii, Allium sativum*
Tranquilizing	*Rauwolfia serpentine*
Anesthetic	*Erythroxylum coca*
Spasmolytic	*Atropa belladona, Hyoscya musniger*
Diuretic	*Phyllanthus niruri, Centella asiatica*
Astringent	*Piper beetle, Abrus precatorius*
Antihelmintic	*Punica granatum*
Cardio tonic	*Digitalis* sp., *Thevetia* sp.
Antiallergic	*Nandina domestica, Scutellaria baicalensis*
Hepatoprotective	*Andrographis paniculata*

Table 1.4 Based on habitat

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

Table 1.5 Based on habit

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

1.9 Goals of using medicinal plants as sources of therapeutic agents:

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

1. To isolate bioactive compounds for direct use as drugs, (E.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastine, Vincristine).

2. To produce bioactive compounds of novel or known origin as lead compounds for semi synthesis to produce molecules of higher activity and / or lower toxicity, (E.g. Metformin, Nabilone, Oxycodone and other narcotic analgesics, Taxotere, Teniposide, Verapamil, and Amiodarone, which are based on Galegine, 9 – tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin respectively).

3.To use agents as pharmacologic tools (E.g. LSD, Mescaline, Yohimbine).

4.To use the whole plant or part of it as a herbal remedy, (E.g. Cranberry, Echinacea, Feverfew, Garlic, Ginkgo biloba). (Fabricant et al, 2001)

1.10 Importance of Medicinal Plants as Drugs

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

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

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

1.10.1 Alkaloids:

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many

alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste .

1.10.2 Flavonoids:

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

1.10.3 Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom tri terpenes in plants. They are found in various plant parts; leaves, stems roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently (Oakenfull and Sidhu, 1990). Saponins also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plants immune system acting as an antibiotic to protect the plant against microbes and fungus.

1.10.4 Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and

insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative.

1.10.5 Glycosides

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

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

Cardiac glycosides acts on the heart. Anthracene glycosides as purgative and for treatment of skin diseases, Chalcone glycoside as anticancer agent. Gentiopicrin, andrographolide, ailanthone and polygalin are used as flavoring agents in many pharmaceutical preparations. Amygdalin has been used in the treatment of cancer, and also as a cough suppressant in various preparations.

1.10.6 Cardiac glycosides

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

1.10.7 Phenolics

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

1.10.8 Tannins

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

1.10.9 Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins. Examples of commonly important monterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C20) are classically

considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes (C30) include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes are amyrins, ursolic acid and oleanic acid. Sesquiterpene (C15) is major components of many essential oils. The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic action.The sesquiterpene lactone, palasonin, has anthelmintic activity, inhibits glucose uptake.

1.10.10 Essential Oils

Essential oils are the odorous and volatile products of various plant and animal species. Essential oils have a tendency evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils. They mostly contribute to the odoriferous constituents or 'essences' of the aromatic plants that are used abundantly in enhancing the aroma of some spices. Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odor.(Farnsworth, et al 2001)

1.11 Medicinal Plants: Natural Antibiotics

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

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

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

1.12 Medicinal Plants: Antimicrobial agents

Medicinal plants have always been considered as a source for healthy life for people. Therapeutic properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural. In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years.

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections . Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and reemerging infectious diseases.

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics.

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

1.13 Medicinal Plants: Potential Antioxidants

In living systems, oxidation is a basic part of the normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated.

Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging. Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body.

There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites. So to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above disease conditions.

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

1.14 Medicinal Plants: Anti-cancer Activity

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

Camptothecin has been effective against a broad spectrum of tumors. Camptothecin is a quinoline based alkaloid found in the bark of the Chinese camptotheca tree. It has been used for psoriasis, leukemia and diseases of liver, gall bladder, spleen and stomach. Even though there are number of synthetic antitumor agents available, efforts are still on to search for effective naturally occurring anti carcinogens that would prevent, slow or reverse cancer development. Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds constitute more than 50% of anticancer agents.(Dong, 2003)

1.15 Use of Medicinal Plant in Bangladesh

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

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

1.16 Natural Sources: A Model for Synthetic Drugs

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

1. Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.

2. Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.

3. Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs.

Though most of the modern medicines are gift of synthetic chemistry, there are still some synthetic drugs where plant constituents act as "lead" (precursor) molecule. Procaine, a synthetic compound, displaces cocaine, isolated from coca leaves, due to its lacking of addiction property.Due to relatively low therapeutic index of procaine, search of new synthetic products lead to synthesis of Lidocaine, tetracaine and dibucaine. The discovery of diosgenin from Mexican Yams (Dioscoria) as a starting material for the synthesis of progesterone decreases the cost of progesterone from 80 U.S. $ per gm to 1.7 U.S. $ per gm. Also lifesaving antibiotic penicillin is synthesized from a natural product 6-aminopenicillanic acid derived from *Penicillium notatum* (Cox and Balick 1994).

1.17 Plant Review

The mangosteen tree is very slow-growing, erect, with a pyramidal crown; attains 20 to 82 ft (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; (9-25 cm) long, (4.5-10 cm) wide, with conspicuous, pale midrib. New leaves are rosy. Flowers, (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. (Bhargava et al, 2011).

1.16.1 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

1.16.2 Taxonomical Classification:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Malpighiales
Family: Clusiaceae
Genus: *Garcinia*
Species: *Garcinia cowa*

1.16.3 Distribution:

Available at different places of Comilla and Chittagong.

1.16.4 Parts Used:

Leaves, barks and fruits.

1.16.5 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. Ethanolic extract of the leaf possesses antibacterial properties. 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. A new compound 1,3,6-Trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)-xanthone has been isolated from stems. (Ghani,2012).

1.16.6 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 chewsticks 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.

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.

1.16.7 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

mangostin, mangosin-e, 6-di-*O*-glucoside, is a central nervous system depressant and causes a rise in blood pressure.

1.16.8 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.

Figure 1.2: Whole Plant of *Garcinia Cowa*

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).

1.16.9 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 recognised 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 atmaturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3- angled seeds.

Figure 1.3: Flower of *Garcinia cowa*

Figure 1.4: Fruit 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,

Figure 1.5: Foliage of *Garcinia cowa*

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

1.16.8 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 15-30g/mL) but not *E. coli* (IC50s 250-500g/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 20.5g/mL and was more potent (higher % inhibition at 1000g/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 g of extract for the leaf and twig extracts respectively. (Ghani, 2012)

1.17 Classes of Compounds Isolated from *G.cowa:*

1.17.1 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.

1.17.2 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 epicambogin. Only one compound, chamuangone, 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). 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, 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.

1.17.3 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; cowaxanthones B (34), C (39), D (42) and E (44) from the fruit; 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 and cowaxanthone from the twig. Most of these xanthones showed interesting biological activities.

1.17.4 Antibacterial activity:

Eight xanthones from the fruit: cowaxanthones B (34) and C (39), 7-O-methylgarcinone E (36), mangostin, mangostin, mangostanin, cowanol and cowanin were investigated for their antibacterial activity against S. aureus and MRSA. Mangostin and mangostanin showed significant activity against these bacteria. 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.

1.17.5 Anti-inflammatory activity:

Eight xanthones: cowaxanthones A (32), B (34), C (39) and D (42),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,mangostin, cowaxanthone, cowanol and cowanin had significant in vitro antimalarial activity against Plasmodium falciparum with IC50 values ranging between 1.5-3.0 μg/mL. Anticancer activity Six xanthones: cowaxanthone, cowanol, cowanin, norcowanin,

3,6-di-Omethyl-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-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 IC50 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. (Barboza et al, 2009).

Chapter 2 : Literature Review

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

The bark extracts of *Garcinia pedunculata* and *Garcinia cowa,* which are 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.(Anushi Sharma et al,2014)

2.2 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*. (Negi et al, 2008)

2.3 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 (IC50) 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 (IC50 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.(Fatma et al, 2009)

2.4 In vitro and in vivo toxicity of *Garcinia cowa* or hydroxycitric Acid: a review

Obesity is one of the pandemic chronic diseases commonly associated with health disorders such as heart attack, high blood pressure, diabetes or even cancer. Among the current natural products for obesity and weight control, Garcinia or more specifically hydroxycitric acid (HCA) extracted from Garcinia has been widely used. The evaluation of the potential toxicity of weight control supplement is of the utmost importance as it requires long term continuous consumption in order

to maintain its effects. Majority of reports demonstrated the efficacy of Garcinia/HCA without any toxicity found. However, a few clinical toxicity reports on weight-loss diet supplements of which some were combinations that included Garcinia/HCA as an active ingredient showed potential toxicity towards spermatogenesis. Nonetheless, it cannot be concluded that Garcinia/HCA is unsafe. Those products which have been reported to possess adverse effects are either polyherbal or multi-component in nature. To date, there is no case study or report showing the direct adverse effect of HCA. The structure, mechanism of action, long history of the use of Garcinia/HCA and comprehensive scientific evidence had shown "no observed adverse effect level (NOAEL)" at levels up to 2800 mg/day, suggesting its safety for use. (Li Oon Chuah et al,2012)

2.5 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. (Jena et al,2005)

2.6 Cytotoxic Properties and Complete Nuclear Magnetic Resonance Assignment of Isolated Xanthones from the Root of *Garcinia cowa*

To isolate compounds from the roots of Garcinia cowa and to evaluated their cytotoxic activity against breast (MCF-7), prostate (DU-145), and lung (H-460) cell lines. Materials and Methods: The ground air-dried root was sequentially macerated with hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and methanol. The DCM soluble extract was fractionated by vacuum

liquid chromatography, column chromatography, and radial chromatography over silica gel with hexane, EtOAc and methanol as eluent in progressively increasing polarity manner; to yield three compounds. Their structures were elucidated based on their spectroscopic data and their comparison with those of the literature. The cytotoxicity of isolated compounds was carried out against human cell lines by 3-(4,**5**-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. The extract was added at various concentrations (0.1, 1, 10 and 100 µg/ ml).The level of cytotoxicity was determined by calculating the level of IC50 that was based on the percentage of the cell death following the 24 h incubation with the extract. Results: Phytochemical study on the roots of G. cowa yielded rubraxanthone (3), cowanine (4) and 1,5-dihydroxyxanthone (5). Compound 4 with an IC_{50} value of 4.1 ± 1.0 µM, 5.4 ± 2.3 µM and 11.3 ± 10.0 µM against MCF-7, H-460, and DU-145. (Dachriyanus et al. 52)

2.7 Cytotoxic Compounds from the Leaves of *Garcinia cowa*

The aims 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.(Wahyuni et al. 006-011)

2.8 Inhibitory effects of the extracts of Garcinia species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents

Twenty-two methanol extracts from different parts of nine Garcinia species were investigated for their ability to inhibit platelet aggregation and low-density lipoprotein (LDL) peroxidation and their total phenolic contents (TPC). The antioxidant activity of the extracts was examined using thiobarbituric acid reactive substances (TBARS) assay with human LDL as the oxidation substrate and their antiplatelet activity in human whole blood was determined by using an electrical impedance method. The TPC of the extracts were measured by the Folin-Ciocalteau method. Among all samples studied, the leaf extract of Garcinia eugenifolia Wall showed the highest inhibitory activity on LDL peroxidation with IC_{50} value of 12.5 μg/ml. The twig extract of Garcinia mangostana Linn. was the most effective sample against platelet aggregation caused by arachidonic acid (AA) with IC50 value of 15.6 μg/ml. The TPC of the extracts varied from 4.4 to 62.8 mg of gallic acid equivalents per g (mg GAE/g). The Pearson correlation analysis revealed that TPC showed moderate positive correlations with antioxidant ($r = 0.30$, $p < 0.05$) and antiplatelet activities (AA-induced, $r = 0.62$, $p < 0.05$; ADP-induced, $r = 0.42$, $p < 0.05$; collageninduced, $r = 0.54$, $p < 0.05$). Thus, it was concluded that the antioxidant and antiplatelet activities of the Garcinia extracts could partly be due to their total phenolic contents.(Jantan and Saputri 58-63).

2.9 Cowaxanthone F, a new tetraoxygenated xanthone, 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**), α-mangostin (**16**), mangostanin (**17**), and cowanol (**18**), were also investigated for anti-inflammatory activity using ethyl phenylpropiolate (EPP)-induced ear edema. Assessment at 30, 60, and 120 min revealed that cowaxanthones B–D (**7–9**), cowanin (**15**), and α-mangostin (**16**) exhibited significant anti-

inflammatory activity when compared to phenylbutazone, while cowaxanthone A (**6**), mangostanin (**17**), and cowanol (**18**) showed less activity.(Kanda et al, 2009)

2.10 Flavanone glucuronides from the leaves of *Garcinia prainiana*

Phytochemical investigation of Garcinia prainiana's leaves led to the isolation of prainianonide (**1**), a new flavanone glucuronide, together with six known compounds: (2*S*)-eriodictyol 7-*O*-β-d-glucuronide (**2**), naringenin 7-*O*-β-d-glucuronide (**3**), (–)-GB-1a (**4**), (+)-morelloflavone (**5**), amenthoflavone (**6**), and friedelin (**7**). Their structures were elucidated by spectroscopic methods. The absolute configuration of **1** was determined by circular dichroism spectroscopy. Compounds **4** and **5** showed significant antioxidant activity.(Saranyoo et al, 2011)

2.11 Utilization of some *garcinia* species in thailand

Garcinia is in the *Clusiaceae (Guttiferae)* family. This genera consists of many species, which are widely used as a source of edible fruits, timber, resin, and various other natural products. Twenty-two *Garcinia* species are reported in Thailand. Some species are well known and used in many ways. *G. mangostana* is one of the best known tropical fruits and is referred to as the 'queen of tropical fruits'. Apart from the aril being consumed as a dessert fruit, the dried fruit rind, which contains tannin and xanthones, is used as a native anti-inflammatory and anti-diarrhea medicine and for treatment of dysentery. Young leaves of *G. cowa* are used as a food additive in many Thai dishes. Fruits of *G. schomburgkiana* are made into a remarkably fine preserve. Gum resin of *G. hanburyii* is used as a potent purgative and for colouring. Young shoots and the mature fruit of *G. xanthochymus* are eaten as vegetables and edible fruits. *G. dulcis* is grown as a fruit tree in southern Thailand. The tall, slender form of *G. thorelii* makes it popular as a source of poles.(Subhadrabandhu et al, 2002)

2.12 Kaennacowanols A–C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*

Three new xanthones, named kaennacowanols A–C (**1**–**3**), along with nineteen known xanthones were isolated from the roots of *Garcinia cowa* Roxb. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **17** and **22** showed good cytotoxicity against KB cell with IC_{50} values of 7.97 and 9.10 μM, respectively. On the other hand, compound **15** showed good cytotoxicity against HeLa cell with IC_{50} value of 9.34 μM. (Kaennakam et al, 2015)

Chapter 3 : Materials and Methods

3.1 Preparation of the Plant Extract for the Experiments

3.1.1 Collection & Preparation of Plant Material:

Garcinia cowa plant was collected in the month of June, 2016 from Comilla. Then proper identification of plant sample was done by an expert taxonomist. The stems of the plant were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried stems were then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.1.2 Washing and Drying of *Garcinia cowa* stems:

At first the plant parts were thoroughly washed with tap water to remove dust, soil, bird's droppings etc within them. The stems were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the parts were dried in hot air oven at 50°C for 2 hours.

3.1.3 Grinding and Storage of Dried Samples:

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered stem was 500 gm which was measured using electronic balance.

3.1.4 Extraction of the Dried Powdered Sample:

The fine powder of *Garcinia cowa stem* was dissolved in 5L petroleum ether and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.5 Filtration of the Extract:

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper was prepared for rotary evaporation.

3.1.6 Evaporation and Condensation of the extracts:

The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator, with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50ºC. It run for 1 hours 10 minutes and the RPM was set 80 for evaporation process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask by using dichloromethane. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4˚C) dry place for further assay.

3.1.7 Principle of a Rotary Evaporator:A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by

other language (e.g., "the sample was evaporated under reduced pressure"). Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

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

Figure 3.1: Drying of extract using Rotary evaporator

3.2 Theory of Phytochemical Screening

Table 3.1 Materials (Reagents and Tools) Used

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.	

3.2.1.1Test Compounds

Pet Ether extract of *Garcinia cowa* stems.

3.2.1.2 Preparation of Sample Solution

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

3.2.2 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 flavanoids:*** A few drops of conc. HClwas 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.

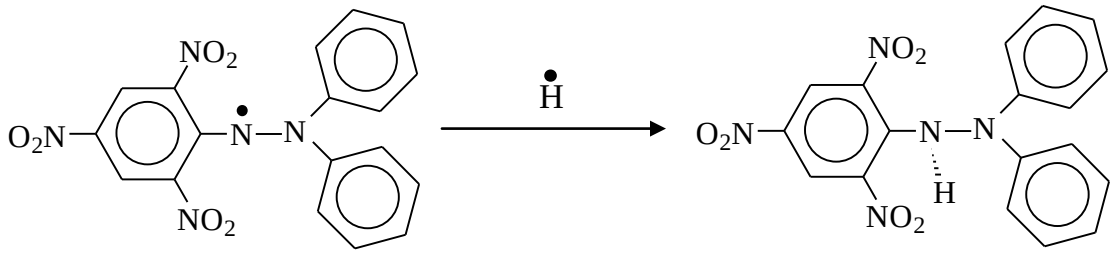
Assessment of In Vitro Pharmacological Property

3.3. Determination of Antioxidant property

DPPH Free Radical Scavenging Assay(Braca *et al.,* 2001)

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**

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

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

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/ extact. The experimental concentrations from the stock solution were prepared by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by 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 (12.5µg/ml)	1 ml	-
6.25	-	1 ml (6.25µg/ml)	1 ml	-

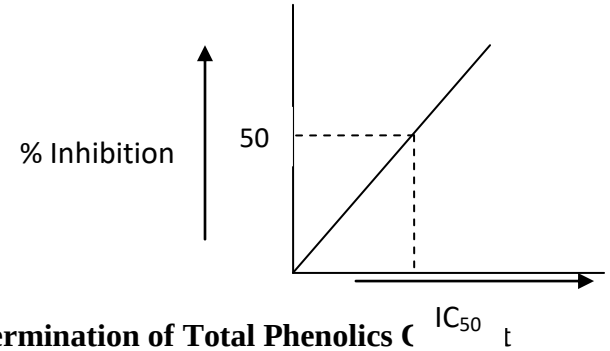
Procedure

- 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

Calculation

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

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.



3.4 Determination of Total Phenolics C

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). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Vinson et al, 2005).

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) $\longrightarrow$

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

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.

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

Concentration	Solution taken	Solution taken	Adjust the	Final volume ml

(µg/ml)	from stock solution (µl)	from others	volume by Absolute ethanol µl	
200	80	-	1920	2
100	-	1ml (200 µl/ml)	1000	2
50	-	1ml (100 µl/ml)	1000	2
25	-	1ml (50 µl/ml)	1000	2
12.5	-	1ml (25 µl/ml)	1000	2
6.25	-	1ml (12.5 µl/ml)	1000	2

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:

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

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 m l m = the weight of crude plant extract in gram

3.6 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).

3.6.1Materials

3.6.1.1Microorganisms

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

3.6.1.2Test 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 3.3 List of Test Bacteria*:*

Gram positiveBacteria	**Gram negativeBacteria**
Bacillus megaterium	*Escherichia coli*
Bacillus subtilis	*Salmonella typhi*
Staphylococcus aureus	*Shigella dysenteriae*
Vibrio parahemolytics	*Salmonella paratyphi*
Sarcina lutea	

3.6.2 Culture Media and Chemicals

- Nutrient agar media
- Ethanol
- Chloroform

Equipments

- Filter paper discs
- Petridishes
- Inoculating loop
- Sterile cotton
- Screw cap test tubes
- Nose-mask and Hand
- Laminar air flow hood
- Autoclave

- Sterile forceps
- Spirit burner
- Micropipette
- Incubator
- Refrigerator

3.6.3 Test Materials

The pet ether extract of *Garcinia cowa stems* were tested against gram-positive and gram-negative bacteria.

3.6.4 Methods

Culture Preparation

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.

Composition of Nutrient agar media (1000 ml)

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

3.6.5 Sterilization Procedure*:*

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. 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.

3.6.6 Preparation of Subculture

In an aseptic condition under laminar air hood cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop tohave 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.

3.6.7 Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial 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

3.6.8 Preparation of Discs

3.6.8.1 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, Amoxycillin (10µg/disc) standard disc was used as the positive control.

3.6.8.2 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.

3.6.8.3 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 laminarhood. Then discs were soaked with 10 µl of solutions of test samples containing 200 µg and 300µg of extract. Then the disks were dried.

3.6.9 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.

3.6.10 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.

Chapter 4: Results and Discussion

4.1 Phytochemical screening of Pet-ether extract of *Garcinia cowa* stem

Table: 4.1 **Phytochemical screening of Pet-ether extract of *Garcinia cowa* stem**

Carbohydrate	Glycosides	Alkaloids	Saponins	Flavonoids	Steroids	Tannins
–	+	+	–	–	–	+

4.2 DPPH free radical scavenging assay of Pet-ether extract of *Garcinia cowa* stems

Table 4.2: Result of absorbance and %of inhibition of pet-ether extract of *Garcinia cowa* stems and ascorbic acid

Serial no	Concentration (ug/ml)	Absorbance 0f Standard	Absorbance of sample	Percentage of inhibition of sample	Percentage of inhibition of ascorbic acid
1	400	0.019	0.243	53.02013	97.53915
2	200	0.028	0.234	49.88814	96.86801
3	100	0.021	0.228	49.66443	95.74944

4	50	0.019	0.229	49.217	82.55034
5	25	0.019	0.230	48.99329	78.07606
6	12.5	0.025	0.224	47.65101	49.88814
7	6.25	0.029	0.210	45.63758	29.75391
8	0	0	0	0	0

4.2.1 Preparation of DPPH Scavenging Activity Curve

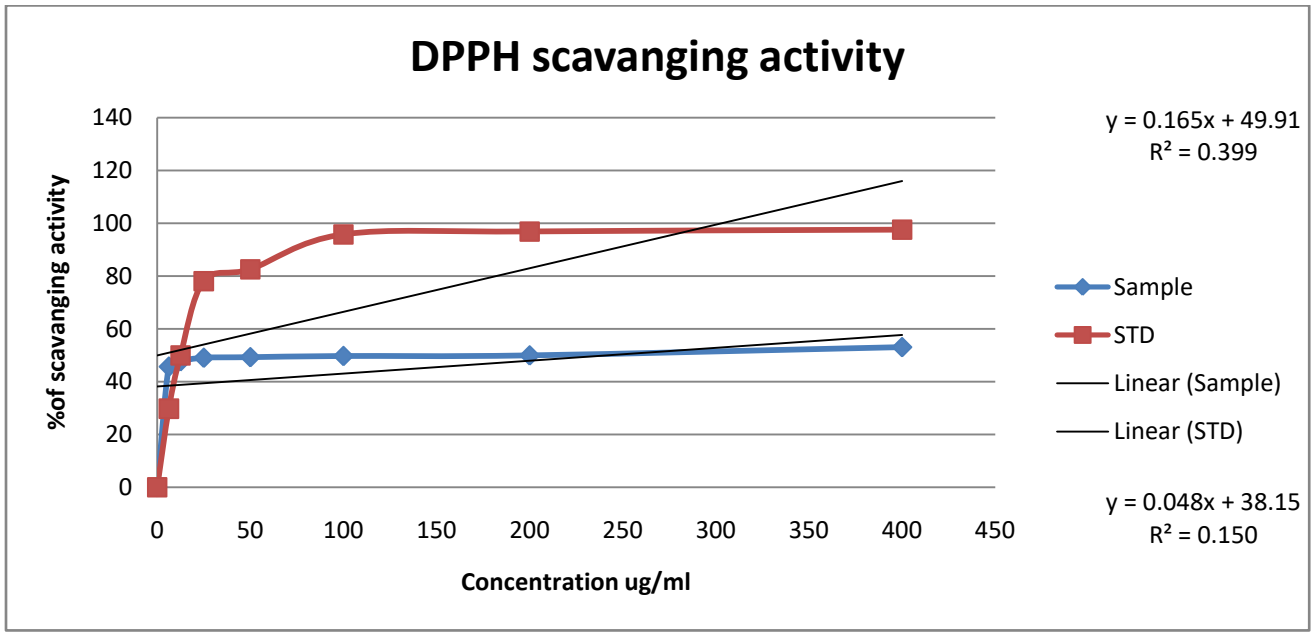


Figure 4.1: DPPH Scavenging Activity of pet-ether extract of *Garcinia cowa* stems.

4.2.2 Result of DPPH test of pet-ether extract of *Garcinia cowa* stem

Table 4.3 **Result of DPPH test of pet-ether extract of *Garcinia cowa* stem**

Pet-ether extract of *G.cowa* stem / Ascorbic acid	**IC50 Value (µg/ml)**	**Regression Line**	R^2 value
Pet-ether extract of *G.cowa* stem	246.875	Y= .048x + 38.15	R^2 = 0.150
Ascorbic acid	0.5454	Y =.165x + 49.91	R^2=0 .399

4.4 Phenolic content of pet-ether extract of *Garcinia cowa* stem

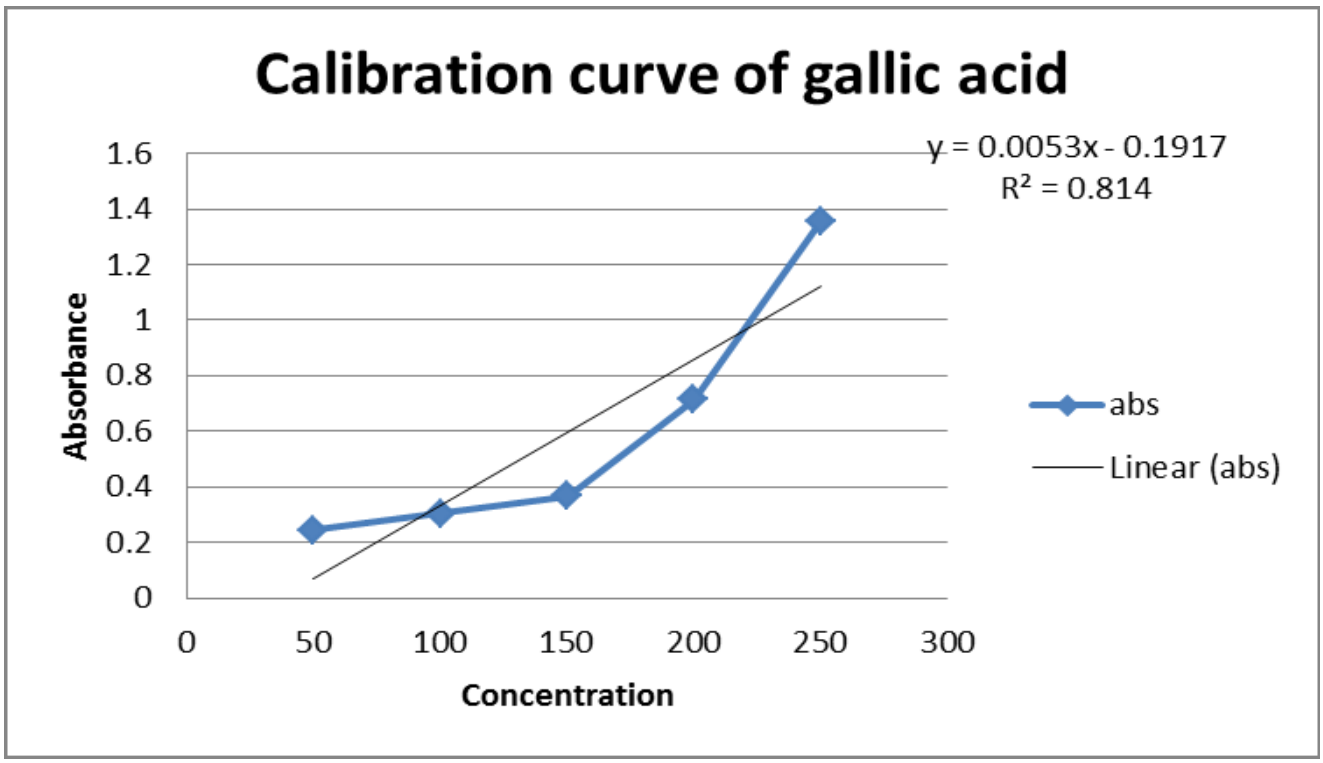


Figure 4.2: Standard Curve of Gallic Aci

Table 4.4 Result of total phenolic content (mg/g) gallic acid equivalent

Serial no	Absorbance of pet-ether extract of *G.cowa* stem cells	Standard equation	Value of x	Mean	Standard deviation	Total phenolic content mg/g (GAE)
1	0.653		168.8			
2	0.673	Y=0.0053x-0.1917	172.8	173.93	5.78	173.93±5.78
3	0.71		180.2			

4.4 Antimicrobial screening of Pet-ether extract of *Garcinia cowa* stems

Table 4.5 : Result of Zone of Inhibition of pet-ether extract of *G.cowa* stems and Kanamycin

Bacterial sample	Pet-stem extract (300ug/disc) zone of inhibition(mm)	Pet-stem extract (600ug/disc) zone of inhibition(mm)	Kanamycin (30ug/disc) Zone of inhibition(mm)
Bacillus megaterium	7	10	30
Bacillus subtilis	7	9	30
Salmonella paratyphi	6	7	30
Salmonella typhi	7	8	30
Vibrio parahemolytics	8	11	30

Staphylococcus aureus	8	12	35
Escherichia coli	10	15	30
Shigella dysenteriae	8	13	35
Sarcina lutea	10	15	30

4.5 Discussion

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

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

Garcinia cowa is a medicinal plant enriched with various chemical constituents having different medicinal activities. This study has shown the phytochemical test and pharmacological investigation on stems of *Garcinia cowa*

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 phenolic content, antioxidant activity and antimicrobial activity of pet-ether extract of *Garcinia cowa* stems. 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 petroleum ether extract of *Garcinia cowa* stems were rich in phytochemical constituents.Such as-Glycosides, Flavonoid, 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 moderate antioxidant as it has potent antioxidant effect. The antioxidant activity was measured by Phytochemical Screening, DPPH and Total Phenol Content tests. IC50 value of DPPH test was 246.875 µg/ml for pet-ether extract of *G.cowa* stem cells. The Total Phenol content is 173.93±5.78 mg/g equivalent to Gallic Acid for petroleum ether extract of *Garcinia cowa* stems.

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.

In the present study the pet-ether extract of *Garcinia cowa* stems showed the activity against *Bacillus subtilis, Shigella dysenteriae, Sarcina lutea, Staphylococcus aureus, Salmonella paratyphi, Vibrio parahemolyticus, E.coli* etc and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The antimicrobial activity of the pet-ether extract of *Garcinia cowa* stem was tested against nine microorganisms. The highest antimicrobial activity was shown against *Escherichia coli* and *Sarcina lutea.* The diameter of the zone of inhibition was 15 mm (600µg/disc) compared to the 30 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc. It showed the moderate activity against

Shigella dysenteriae, Vibrio parahemolyticus and *Bacillus megaterium, Staphylococcus aureus* . In case of 300µg/disc, the highest zone of inhibition of *Garcinia cowa* was 10 mm for *Escherichia coli* and *Sarcina lutea* where the zone of inhibition of Kanamycin was 30 mm. It showed very little activity against *Salmonella paratyphi, Salmonella typhi, Bacillus subtilis.* The test results vary may be due to some experimental error.

So, the petroleum ether extract of stems of *Garcinia cowa* 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.

From previous work done with different parts of *Garcinia cowa* plant, it is known that its bark extract showed anti-oxidant and anti-platelet activity due to having phenolic content which prohibit low density lipoprotein peroxidation. Different organic acids and chemical constituents were also found like xanthone, alkaloid, flavonoid, tannins etc. Some of them gave antibacterial and antimicrobial activity. Leaf extracts of *Garcinia cowa* showed anti-inflammatory activity. Similarly, on this research work I found some phytochemical constituents which facilitates moderate antioxidant and antimicrobial properties from the petroleum ether extract of stems of *Garcinia cowa*.

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

Chapter 5 : Conclusion

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

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 emerging 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 and antimicrobial activity. There are some established research reports regarding the phytochemical and antimicrobial properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

Chapter 6 : References

Ahmed, Azam, 2011, "Phytotoxic, Antibacterial and Haemagglutination activities of the aerial parts of Mangosteen*.",*African Journal of Biotechnology, vol. 10, pp. 97-102

Aggarwal, Surh and Shishodia, (2007). The molecular targets and therapeutic uses of curcumin in health and disease. New York, NY: Springer.

Anushi, Joseph, Singh. Food Sci Technol. 2014 Aug; 51(8): 1626–1631. Published online 2014 Apr 24.last visited on 10th October,2017.

Balunas and Kinghorn. (2005). Drug Discovery from Medicinal Plants. Life Sciences, 78, 431-441.

Borsani & Abelli, 2004, "Guidelines on Developing Consumer Information on Proper Use of Traditional, Complementary and Alternative Medicine", World Health Organization, WHO,Geneva.

Chopra, Hunda and Kapur. (1982). Chopra's indigenous drugs of India. 3rd edition. Calcutta: India: Academic publishers, pp.1-1

Dachriyanus, Hamidi et al. "Cytotoxic Properties And Complete Nuclear Magnetic Resonance Assignment Of Isolated Xanthones From The Root Of Garcinia Cowa Roxb." Pharmacognosy Magazine 12.45 (2016): 52.

Das and Choudhury. (2012). Ethnomedicinal uses of some traditional medicinal plants found in Tripura, India. Journal of Medicinal Plants Research, 6, pp.4908-4914.

Duke et al. (1993). Medicinal plants and the pharmaceutical industry (pp. 664–669). New York, NY: In New Crops.

Fabricant and Farnsworth. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. 2nd ed. Environmental Health Perspectives, pp.vol.109, pp.69-75.

Galatea, (2016). Pros and Cons of Natural & Synthetic Drugs. [Online] Available from: http://www. mynutrishop.com/pros-cons-natural-synthetic-drugs (Accessed: 10th October, 2017)

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

Grene and Depew, (2004). The philosophy of biology. Cambridge: Cambridge University Press, p.11.

Huff, (2003). The rise of early modern science. Cambridge [England]: Cambridge University Press, p.218.

"In Vitro And In Vivo Toxicity Of *Garcinia cowa* Or Hydroxycitric Acid: A Review | Read By Qxmd." Readbyqxmd.com. N.p., 2017. Web. 10 Oct. 2017.

Jantan, Ibrahim, and Fadlina Chany Saputri. "Benzophenones And Xanthones From Garcinia Cantleyana and Their Inhibitory Activities On Human Low-Density Lipoprotein Oxidation And Platelet Aggregation." Phytochemistry 80 (2012): 58-63.

Jena, Bhabani, Jayaprakasha and Kunnumpurath. "Organic Acids From Leaves, Fruits, And Rinds Of *Garcinia cowa*." Journal of Agricultural and Food Chemistry 50.12 (2002): 3431-3434. Web.

Jalalpour et al(2012). Ginkgoales: Fossil Record. [online] Available at: http://www.ucmp.berkeley.edu/seedplants/ginkgoales/ginkgofr.html

Kaennakam, Sutin, Pongpun Siripong and Santi Tip-pyang,2015. "Kaennacowanols A–C, Three New Xanthones And Their Cytotoxicity From The Roots Of *Garcinia Cowa*." .Vol-102. Page:171-176. http://www.sciencedirect.com/science/article/pii/S0367326X15000623

Kanda Panthong, Nongporn Hutadilok-Towatana, Ampai Panthong. Canadian Journal of Chemistry, 2009, 87 (11), 1636-1640, 10.1139/V09-123https://doi.org/10.1139/V09-123

Kelly et al (2009). History of medicine. 12th ed. New York: Facts on file, pp.29-50.

Kremers and Urdang. (1986). Kremers und Urdang's history of pharmacy. Madison: American Institute of the History of Pharmacy, p.40

KiÌrtikar, Blatter, Sing, (1980). Indian medicinal plants. 2nd edition. Dehra Dun: Bishen Singh Mahendra Pal Singh, p.420.

Maridass & Britto , (2008). Origins of Plant Derived Medicines, Ethnobotanical Leaflets, vol-12, pp 373–387.

Md Lip Jabit , Fatma Sri et al, 2009. "Citotoxic and nitric oxide inhibitory effect of methanol extract of *Garcinia cowa*". http://www.tandfonline.com/doi/full/10.3109/13880200902973787

Negi, Jayaprakasha and Jena. "Antibacterial Activity Of The Extracts From The Fruit Rinds Of *Garcinia Cowa* And Garcinia Pedunculata Against Food Borne Pathogens And Spoilage Bacteria." N.p., 2017.

Pelton, (2000). Gingko biloba. Natural Choices Informed Decision, 19, pp.1-9.

Quack, (2012). Disenchanting India. New York: Oxford University Press, p.213.

Robson and Baek, (2009). The engines of Hippocrates. Hoboken, N.J.: John Wiley & Sons, p.50.

Rashid, Rahman, Wahab and Chowdhury, (2008). Antimicrobial Activity of Some Indigenous Plants of Bangladesh. Dhaka Univ. J. Pharm. Sci, 7(1), pp.23-26.

Sumner and Judith, (2000). The Natural History of Medicinal Plants. *Timber Press*, pp.17-18.

Schimmel, Richel and Guchelaar, 2004. Cardiotoxicity of cytotoxic drugs. Cancer treatment reviews, 30(2), pp.181-191.

Singh, (2010). History of science, philosophy and culture in Indian civilization. New Delhi: Project of History of Indian Science, Philosophy and Culture (PHISPC).

Shang, Huwiler, Nartey, Juni and Egger (2007). Placebo-controlled trials of Chinese herbal medicine and conventional medicine comparative study. International Journal of Epidemiology, 36(5), pp.1086-1092.

Shariff, Sudarshana, Umesha and Hariprasad, (2006). Antimicrobial activity of Rauvolfia tetraphylla and Physalis minima leaf and callus extracts. African Journal of Biotechnology, 5, pp.946-950.

Saranyoo Klaiklay, Yaowapa Sukpondma, Vatcharin Rukachaisirikul, Nongporn Hutadilok-Towatana, Kanokphorn Chareonrat . Canadian Journal of Chemistry, 2011, 89(4): 461-464, https://doi.org/10.1139/V10-168

Wahyuni, Fatma et al. "Cytotoxic Compounds From The Leaves Of Garcinia Cowa Roxb.." Journal of Applied Pharmaceutical Science (2015): 006-011. Web.

Wells, (2009). Longman pronunciation dictionary. 9th ed. Harlow: London: Pearson Longman.