Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* stem

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

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DECLARATION BY THE CANDIDATE

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

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation entitled, "**Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* stem"** is a research work carried out by, Farhana Sharmin Shurovi (ID: 2014-1-70-023) in 2017, under the supervision and guidance of me, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy. The thesis has not formed on the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation entitled, "**Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of *Garcinia cowa* stem**" is a research work carried out by Farhana Sharmin Shurovi (ID: 2014-1-70-023), under the supervision and guidance of Ms. Nazia Hoque, Assistant Professor, Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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Dedication

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ABSTRACT

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

The aim of the present study was to evaluate the antioxidant and antimicrobial activity of dichloromethane extract of *Garcinia cowa*. The antioxidant activity was measured by DPPH and total Phenol test. The IC_{50} values of DPPH test was 53.404 μg/ml for dichloromethane extract of *Garcinia cowa* stems. The Total Phenol content was 173.93 ± 5.87 mg/g equivalent to Gallic Acid for methanol extract of *Garcinia cowa* stem. By determining antioxidant property, the result suggests that the plant extract which have been tested possesses antioxidant property. The antimicrobial activities of dichloromethane extract of *Garcinia cowa* stem were tested against ten microorganisms by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The dichloromethane extract of *Garcinia cowa* stem showed good antimicrobial activities (6 mm-16mm) against the microorganisms. Dichloromethane extract of *Garcinia cowa* stem showed highest activity against *Sarcina lutea* and *Pseudomonas aureus.Moderate* activity against *Vibrio parahemolyticus*, *E.coli, Bacillus megaterium, Staphylococcus aureus, Shigella dysenteriae.*

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

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Chapter 1

Introduction

1.1 General introduction

Medicines are chemicals or compounds used to cure, halt, or prevent disease; ease symptoms; or help in the diagnosis of certain illnesses. Advances in medications have enabled doctors to cure many diseases and save lives. These days, medicines come from a variety of sources. Many were developed from substances found in nature, and even today many are extracted from plants. For example, one medicine that is used to treat certain cancers comes from the Pacific yew tree. Some medicines are produced in a laboratory by mixing together a number of chemicals. Others, like penicillin, are byproducts of organisms such as fungus. And a few medicines are even biologically engineered by inserting genes into bacteria that make them produce the desired substance.

The word "herb" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now a days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavanoid, medicine or perfume and also in certain spiritual activities.Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

When we think about taking medications, we often think of pills. But medications can be delivered in many ways, such as:

- liquids that are swallowed (like cough syrup)
- drops that are put into ears or eyes
- creams, gels, or ointments that are rubbed onto the skin
- inhalers (like nasal sprays or asthma inhalers)
- patches that are stuck to skin (called transdermal patches)
- tablets that are placed under the tongue (called sublingual medicines; the medation is absorbed into blood vessels and enters the bloodstream)
- injections (shots) or intravenous (inserted into a vein) medications

No medicine can be sold unless it has first been approved by the U.S. Food and Drug Administration (FDA). The manufacturers of the medication perform tests on all new medicines and send the results to the FDA .The FDA allows new medicines to be used only if they work and if they are safe enough. When a medicine's benefits outweigh its known risks, the FDA usually approves the sale of the drug. The FDA can withdraw a medication from the market at any time if it later is found to cause harmful side effects.

Plants have been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems such as Ayurveda an Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes.
In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favor of rugs. Almost one fourth of pharmaceutical drugs are derived from botanicals.

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

Some medicines may cause problem if it take them with other medicines. This is why it is important to tell the doctor and the pharmacist about all the medicines is taking. Some medicine can cause problem if we take them incorrectly .Call the doctor if the situation is getting worse.(Jennifer Alinio,2007).

1.2 Medicinal plant

WHO consultative body of medicinal plants has formulated a definition of medicinal plants in the following way "A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs. WHO consultative body of medicinal plants has formulated a definition of medicinal plants in the following way "A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs.

Plants and man are inseparable. Plants existed on the earth in the geological past form the early history of the earth. The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. From the early stages of human civilization, plants, especially medicinal plants have played a pioneering role for the welfare of human beings. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores of people of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75-80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects.

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. When a plant is designated as "medicinal", it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes. There are a huge number of medicinal plants. In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of the world's flowering plant species have been used medicinally.

Sometimes the figure of 70,000 medicinal plant species is cited, but this includes many algae, fungi, and micro-organisms that are not really plants as the word is understood by botanists. In any event, there is no other category of plants useful to man (with the possible exception of ornamental plants) that includes so many species, and the question naturally arises why such a staggering number of plants have useful medicinal properties. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines. A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients .The association of humans and animals with plants obviously originated with the beginning of life on earth, when plants supplied much of the shelter, oxygen, food and medicine needed by higher life forms. Overtime and with the beginning of societies, human learned to recognize and categorize plant materials suited for use in meeting the necessities of life. Of these necessities, the use of herbs and herbal extracts for their healing powers can be traced to earliest of myths, traditions and writings used to codify those plants that can ease pain and treat diseases.

The evolution of these plant-based medicine systems, primarily based on plants within a local area, produced the well-known traditional medicine systems, the Ayurvedic and Unani of the Indian subcontinent, the Chinese and Tibetan of other parts of Asia, the Native American of North America, the Amazonian of South America and several local systems within Africa.

According to World Health Organization (WHO), about 70 percent of the world’s population relies on plants for their primary health care and some 35,000 to 70,000 species has been used as medicaments, a figure corresponding to 14-28% of the 250,000 plants species estimated to occur around the world, and equivalent to 35-70% of all species used world-wide. In today’s global market, more than 50 major drugs originated from tropical plants .From about 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential. The chemical and biological diversity of plants represent a potentially limitless renewable source for the use in the development of new pharmaceuticals. (Sofowora, 1982)

1.3 Plants as a Basis of Some Important Drugs

Higher plants have been used as a source of drugs by mankind for several thousand years. In fact, ancient man was totally dependent on green plants for his day-to-day needs of medicaments. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. With the advancement of synthetic organic chemistry most of the active constituents of plants used in medicine were synthesized. At one time it was thought that ultimately all the plant drugs would be obtained from synthetic sources.

However, in spite of phenomenal progress in the development of new drugs from synthetic sources and the appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw materials for some of the most important drugs. Although data are not available for all countries, a study carried out in the United States by Farnsworth and his colleagues between 1958 and 1980 indicated that although the number of prescriptions issued by community pharmacies in the United States increased considerably, the percentage of prescriptions containing one or more plant product remained constant at a figure of 25%. It has been found that in highly developed countries like the United States more than 100 chemical constituents of definite structure derived from 41 species of plants were used in modern medicine. It has also been estimated that in addition to these active constituents, more than 96 crude extracts were also used in the United States. (Fari A.,et al, 2000).

1.4 Medicinal plant in Bangladesh

Bangladesh there are about 297 Unani, 204 Ayurvedic and 77 Homeopatheic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi– processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations. The market value of drugs produced by these industries from medicinal plants is about Tk. 300 crores. (The Daily Jugantor, 21 June, 2003).Besides, village Kobiraj, street Vendors and Tribal people also use a large number of medicinal plants for the treatment of various diseases. There is no actual figure how many medicinal plants are used in Bangladesh. Chowdhury at SAARC workshop (held on 16-18 June, 2002) gave a brief idea about the amount of medicinal plants used annually in Bangladesh.

A few of them are mentioned here: Ashwagondha (Withania somnifera)- 56,000 kg, Anantamul (Hemidesmus indicus)- 50,000 kg, Kurchi (Holarrhena antidysenterica)-1,00,000 kg, Gulancha (Tinospora cordifolia)- 127,000 kg. According to Hamdard Laboratories (WAQF), in Bangladesh the annual demand for a few medicinal plants are- Satomuli (Asparagas racemosus)– 800 tons, Sarpagondha (Rauvolfia serpentina)– 1,000 tons, Ghritokumari (Aloe vera)– 24,000 tons, Kalomegh (Andrographis paniculata)– 1,000 tons (Hassan, 2003). Every year Bangladesh imports a large quantity raw materials belonging to of medicinal plants mostly under the banner of spices and spends more than 64 crores Taka annually for this purpose. Ironically, 70% of this imported raw materials can be met from the indigenous sources from Bangladesh (Begum, 2003)

Table 1 : Common medicinal plant in Bangladesh

Common name	Botanical name	Source of drug	Uses
Nayantara	*Catharrantus roeses*	Leaves	Cancer,Insomnia, Diabetisblood pressure
Sarpagandha	*Rauvolfia Serpentain benth*	Roots	Insomnia,Brain disorder
Ghritkumari	*Aloe indica*	Leaves	Piles,burns,Jaundice
Lajjabati	*Mimosa pudica*	Whole plant	Blood purification, piles
Patherkuchi	*kalanchoe pinnata*	Leaves	Augh,Lung infection, Wounds
Pudina	*Menthe arventsis*	Whole plant	Indigestion, Stomach disorder
Shimul	*Bombex ceiba*	Roots,Leaves, Barks,Flower, Fruits	Fever,pox,Diarrhea, Dysentery

Basak	*Adhatoda vasica*	Roots,leaves,Flower	Cough,Asthma
Sharisa	*Brassica napus*	*Leaves,Seeds*	Fever ,Common cold
Dhutara	*Dutura metel*	Roots,Leaves,Seeds	Anaesthetia,Pain, Asthma
Nisinda	*Vitex negunda*	Leaves,Barks	Weakness, Headache
Helencha	*Enhydra fluctans*	Whole plant	Nutrition, Skin disorder,Blood purification

1.5 Maintaning Problem

Bangladesh has very rich in Bio-diversity. It has more than 500 medicinal plants species (Yusuf et al., 1994). An alarmingly populous, but size-wise a very small country is rather unique in having diversified genetic resources in a wide range of habitats. Increasing population pressure and multifarious anthropogenic activities on the natural ecosystems are posing severe and serious threats to once dense and rich genetically diversified plant communities of this country. Loss of habitats from the wild forests as well as from the village groves, cultivated plains and wild lands are quite common in this country. A broad genetic base has been replaced by a narrow one, and the old genetic diversity is disappearing both inside and outside of the ancient gene centers. This trend is inevitable with the need for highly efficient and uniform cultivars in advanced and sophisticated farming systems. At present, we have no real protected area for natural genetic resources and also have no specific practical policy on conservation of biodiversity. Although there are several gene banks having limited facilities to preserve some economic crops like rice, jute, wheat, pulses etc in Bangladesh, but there is no centralized organization to maintain germ plasms of the wild relatives for agriculture, horticulture, medicinal and economically less important forest species. Bangladesh Agricultural Research Council (BARC) is very worried about this. (Begum, 2003)

1.6 History of medicinal plant

The term ethno-botany is a compound word of ethnology and botany. While the ethnology is the scientific study and comparison of human races and culture, the latter deals in the scientific study of plants and their structure; therefore one can put Ethno-botany as the scientific study of the interrelation between the human and plants. The term ethno-botany was coined by John W. Harsberger in 1896 (Davis EW, 1995) and was considered as the art of collection of useful plants by a group of people and the description of the uses of plants. Ford developed the science of ethno-botanical study (Ford RL, 1978) and included the understanding of knowledge systems through the use of anthropological methods. (Shengji P, 2002).

Over the last century, ethno-botany has evolved into a scientific discipline that focuses on the people- plant relationship in a multidisciplinary manner, incorporating not only collection and documentation of indigenous uses but also ecology, economy, pharmacology, public health, and other disciplines (Gomez-Beloz A,2002). Presently, ethno-botany has become increasingly valuable in the development of health care and conservation programs in different parts of the world (Balick MJ, 1996). Ethno-botanical studies that explore and help to preserve knowledge are therefore urgently needed before traditional folklores are lost ever (Chaudhary RP, 1998).

Ethno-medicine, a branch of ethno-botany, is a set of empirical local practices embedded in the indigenous knowledge of a social group often transmitted orally from generation to generation (Bussmann RW, Sharon D ,2006) with intent to understand social, cultural, and economic factors (Bhattarai NK, 1992) influencing health problems and to overcome such problems. It is a suitable source of information regarding useful medicinal plants that can be targeted for sustainable domestication and management (Njoroge GN et al, 2004). Ethno-botany and ethno-medicine as sciences addressing indigenous knowledge and practices therefore are now very important for establishing management programs. (Cohen JI et al, 1991).

Native Americans used plants as medicines for centuries and some anticancer drugs that are currently available to treat various cancers are derived from plants in North America such as the Pacific yew tree *(Taxus brevifolia*) from which the anticancer drug paclitaxel (Taxol® , 1) is derived .African traditional medicine has also been practiced for many centuries and it has diverse medical treatments for different diseases but it was poorly recorded..A considerable number of drugs appeared in the second half of the 20th century that can treat diverse diseases as a result of the advancement in pharmaceutical science and the establishment of pharmaceutical companies. Although both synthetic and natural product-based drugs are currently used in curing diseases, numerous drugs in clinical use today are based on plant-derived natural products or their analogues. (Chowdhury, et al,2008).

1.7 Plant purposes in Human History

Plants have also been used in the production of stimulant beverages (e.g. tea, coffee, cocoa, and cola) and inebriants or intoxicants (e.g., wine, beer, and kava) in many cultures since ancient times, and this trend continues till today. Tea (*Thea sinensis*) was first consumed in ancient China (the earliest reference is around CE 350), while coffee (*Coffeaarabica*) was initially cultivated in Yemen for commercial purposes in the 9th century. The Aztec nobility used to consume bitter beverages containing raw cocoa beans (*Theobroma cacao*), red peppers, and various herbs. Nowadays, tea, coffee, and cocoa are important commodities and their consumption has spread worldwide. The active components of these stimulants are methylated xanthine derivatives, namely caffeine, theophylline, and theobromine, which are the main constituents of coffee, tea, and cocoa, respectively. The most popular inebriants in society today are wine, beer, and liquor made from the fermentation of fruits and cereals. Wine was first fermented about 6000−8000 years ago in the Middle East, while the first beer was brewed around 5000−6000 BCE by the Babylonians. The intoxicating ingredient of these drinks is ethanol, a by-product of bacterial fermentation, rather than secondary plant metabolites. Recent studies have shown that a low to moderate consumption of red wine is associated with reduction of mortality due to cardiovascular disease and cancer. (Begum, 2003)

1.8 History of Use of Traditional Medicine

The human race has been struggling along for making his existence safer and sounder as much as possible, trying to avoid and remove any risks which can threaten our health ever since the dawn of our history. An intense desire to lead a safe and sound life is the source of all kinds of civilizations, studies, and cultural activities of men.

How to keep oneself healthy free from disease has been the prime concern to the human race from the very beginning of his existence on the earth. Plants having medicinal virtues are one of the most typical examples which have been utilized by us from the very beginning of our existence. The history of the application of medicinal plants for alleviating disease has its origin in the ceaseless efforts of the most primitive man of the remote past

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Plants that possess therapeutic properties or beneficial pharmacological effects on animal body are generally classified as Medicinal plants. These medicinal plants have been used in traditional folk medication as efficacious remedies for hundreds of years. In Bangladesh, more than 1000 floral species out of 5000 are possibly used in the various practices of traditional medication. The indigenous medicinal plants utilized have been increasing in number, still today, with the discovery and introduction of newer plants. Today, in the traditional medication system, nearly every plants and herbs growing in the country have been assumed that they carry some medicinal virtues, and that are used in the preparation of medicine either as principle therapeutic agent or necessary associate (recipient) to increase the potency of the main agent as well as to make it more stable. The estimate says that more than 1000 metric tons of medicinal plants are required by the industries involved in the manufacture of traditional medication in Bangladesh (Mia & Ghani, 1990).

1.9 Unani

Unani-tib 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 IbnNafis.

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.Mughal Empire. AlauddinKhilji had several eminent Unani physicians (Hakims) in his royal courts. In the coming years this royal patronage meant development of Unani practice in India, but also of Unani literature with the aid of Indian Ayurvedic physicians. (Philosophy and Culture, New Delhi, 2001).

1.10 Ayurvedic

Ayuredic 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. AlauddinKhilji had several eminent Unani physicians (Hakims) in his royal courts.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)

1.11 Traditional Chinese Medicine (TCM)

Emperor's Inner Canon and the Treatise on Cold Damage, as well as in cosmological notions such as 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).

The doctrines of Chinese medicine are rooted in books such as the Yellow yin-yang and the five phases. Starting in the 1950s, these precepts were standardized in the People's Republic of China, including attempts to integrate them with modern notions of anatomy and pathology. In the 1950s, the Chinese government promoted a systematized form of TCM.
TCM's view of the body places little emphasis on anatomical structures, but is mainly concerned with the identification of functional entities (which regulate digestion, breathing, aging etc.).

While health is perceived as harmonious interaction of these entities and the outside world, disease is interpreted as a disharmony in interaction. TCM diagnosis aims to trace symptoms to patterns of an underlying disharmony, by measuring the pulse, inspecting the tongue, skin, and eyes, and looking at the eating and sleeping habits of the person as well as many other things. (Steven Novella, 2012).

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. There is a lack of existing cost-effectiveness research for TCM. Pharmaceutical research has explored the potential for creating new drugs from traditional remedies, but few successful results have been found. A Nature editorial described TCM as "fraught with pseudoscience", and said that the most obvious reason why it hasn't delivered many cures is that the majority of its treatments have no logical mechanism of action, yet proponents argue that it is because research has missed key features of the art of TCM, such as the interactions between different ingredients. (Shang, et al. 2007)

1.12 Characteristics of Medicinal plants

Medicinal plant have many characteristics when used as a treatment, as follows:

1.12.1 Synergic Medicine

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

1.12.2 Support or official medicine

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

1.12.3 Preventive Medicine

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

1.13 Importance of Medicinal Plants

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

The efficacy of some herbal products is beyond doubt, the most recent examples being Silybum marianum (silymarin), Artemisia annua (artemesinin) and Taxus baccata (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, Ginkgo biloba for tinnitus, Hypericum perforatum is a reputed remedy for depression. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herbRecently research has supported biological activities of some medicinal herbs. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease. Allamanda cathratica [allamandin], Elephatopus elatus [elephantpoin], Helenium autmnale [helenalin] Vernonia hymenlepis, Heliotropium indicum [Indicine-N-oxide], Daphne mezereum (mezerien) and Stereospermum suaveolans [laphacol] are medicinal plants that have shown significant tumor inhibiting effect.

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

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

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

Croton sublyratus [plaunotol] has potent and wide spectrum anti peptic ulcer action. A number of plant derivatives have shown anti-Aids activity. Ancistrocladus korupensis [michellamine-b], Caulophyllum langigerum [calanolide-a], Caulophyllum teymanicostatolide-a], Homalanthus nutans [prostratin], Conospermum sp [concurvone] are the medicinal herbs from African countries that are being employed in research for finding a suitable cure for Aids.

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

The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to photochemical screening for the detection of various plant constituents.With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60%of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases. (Singh, N,et al, 2016).

1.14 Scientific Basis of Herbal Drug

Herbal drug is often criticized as non-scientific, inactive and erroneous medicine. But phytochemical and biological investigation proves its medicinal value and therapeutic utility. Traditional medicines that are used topically to treat skin disease contain tannin. Tannin is chemical having antiseptic and astringent property. When it is used topically it reacts with the proteins on infected area to produce a thin but strong barrier. This layer protects the infected area from micro-organism. Besides, tannin has antibiotic property. So it is said that there is no basic difference between herbal drug and allopathic medicine.(Ghani, 1998).

1.15 Rationale of Herbal Drug Research

Special Reference to Bangladesh Most of the people of our country have no or little access to allopathic medicine due to their uncompromisable low income in respect of high cost of allopathic medicine. A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% if people suffering illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mollahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in a crude and substandard form, by our people. The use of such crude and substandard herbal drug is dangerous and may threaten public health. Thus the analysis of plants for exploring the bounty of chemical entities and their biological screening is the current need for standardization of herbal medication.

Since Bangladesh is a country of low economic growth, a proper health care system can be established by supplying low cost medicines to its population. This may be only possible by utilizing our natural resources of medicinal plants and their constituents. So, scientific exploration and standardization of these potential crude drugs is an urgent need to revolutionize our drug sector.Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. During the last five years Bangladesh has spent more than 1500 crore Taka for importing chemicals, raw materials and semi-processed drugs of plant origin from neighboring and other countries and this trend is growing upwards day by day. This huge foreign exchange can be saved if the indigenous medicinal plants or its semi processed products are utilized by the manufacturer to satisfy their need (Ghani, 1998).

1.16 Necessity of Drug Development from Plant Sources

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role. Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents. So the application of herbal drug in crude form may be ineffective or may cause a toxic reaction. Vincristine, a prominent anticancer drug, was developed from periwinkle plant (Vincarosea) which was formerly prescribed for treating diabetes. The efficient hypotensive drug, reserpine, was developed from Rauwolfia serpentine which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN et al., 1982). Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from Ammi visnaga which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Ghani, 1998).

1.17 Medicinal plant classification and benefits of some medicinal plant

Trees and plants are very helpful for food, house, and clothes; apart from this, these are very helpful for medicinal uses. There is no such tree/plant in the world, which are not having medicinal qualities. Plant's root, leaf, flower, fruit, seed, skin, & stem are very helpful for medicinal uses. 85 percentage of Ayurvedic medicinal components were collected from forests and jungles. Some of the important medicinal plants are Amla, Ashwagandha, Plectranthus barbatus, Safed Musli, & Sarpagandha.

1. Amla (Emblica Officinalis)

Figure 1 : Amla

Botanical Name: Emblica Officinalis
Family: Euphorbiaaceae
Height: Average
Age: 40-50 yr
Flower: Greenish Yellow
Fruit: Round shaped; green or yellowish Green; three seeds in each fruit.
Leaf: Small & straight.

Medicinal quality

-Rich with Vitamin 'C' (60-70%), rest with Iron, Calcium, Phosphorus, Tannin, Fat etc.

Medicinal uses

-Amla cures Diabetes, Gastric, Blindness, Heart disorder, Skin disease, Stomachache, etc.
-Used for Diarrhea, Indigestion, Cold, Infection, Jaundice, etc.
-Used as pickles, drinks and other eatables.

2. Plectranthus barbatus (Coleus Forskohlii)

Figure 2: plectranthus barbatus

Botanical name:Coleus forskohlii

Family: Lamiaceae

Height: Nearly 2 – 2.5 feet

Root: Thick & small as Radish

Stem: Round and Plain

Leaf: Thick & Egg shaped

Flower: Blue & Violet, as Tulsi flowers

Medicinal quality

- Root and leaf are very therapeutic.
- Forskolin is the main medicinal standpoint of this.

Medicinal uses

- Leaf juice helps to cure cough, cold and fever in babies.
- Cures heart diseases, chest pain, etc.
- Used as medicine for urine diseases, genial diseases, eye disease, bronchitis etc.
- Root is used for kidney stone, cancer etc.

3. Sarpagandha (Rauwolfia Serpentina)

Figure 3: sarpagandha

Botanical name: Rauwolfia Serpentina

Family: Apocynaceae

Height: Three feet

Flower: White & Rosy colored

Fruit: Small & round shaped

Medicinal quality

- The root is very helpful. There are 30 types of alkaloids present in skin of its root. The average alkaloid present in its root is 0.3 to 3.
- Reserpine alkaloid helps for high blood pressure.
- Other alkaloids present in it are roulfinine, rescinnamine, serptinine, yohimbine, ajmaline, etc.

Medicinal uses

- Helpful for high blood pressure and mental disorder
- Cures cough and Vata
- Useful against bites of poisonous insects/serpents.
- Reliefs from pain and burning.

4. Safed Musli (Chlorophytum Borivilianum)

Figure 4: Safed musli

Botanical name: Chlorophytum Borivilianum

Family: Liliaceae

Leaf: Light yellow, 1.5' long and 1/2"wide

Stem: comprises with root; not visible

Medicinal quality

- Contains nearly 39-42 % alkaloids, 0.5% protein, 2-4 % saponin, 40-45 % polysaccaroids
- Calcium and salt is also present in it

Medicinal uses

- Controls high blood pressure, joint pain, diabetes, impotency, etc
- Cures Vata, Pita, female diseases, heart diseases etc. (Naresh,2011)

1.18 Families of Medicinal Plants

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

A. Medicinal plants of the Compositae family

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

B. Medicinal plants of the Labiatae family

A very important medicinal plant family is the Labiatae family, also known as the mint family. Plants in this family are herbs or shrubs often with an aromatic smell. They are common in the Mediterrane6an countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Some examples from this family include horehound, lavander, balm, micromeria, the mints, thyme and rosemary. The lavander is a term given to a group of plants that have similar shape and properties. In Spain there are several species and subspecies.L. multifida L.

C. Medicinal plants of the Umbelliferae family

The Umbelliferae or carrot family consists of plants with a characteristic umbrella-arranged fruit. These plants usually produce an essential oil, an asset to survive during the hot summer days. In fact the oil has a cooling effect on the plant. Some examples from this family include bullwort (Ammi majus),

D. Medicinal plants of the Leguminosae family

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

E. Medicinal plants of the Rosaceae family

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

F. Medicinal plants of the Rutaceae and Solanaceae families

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

G. Medicinal plants of the Cruciferae family

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

H. Medicinal plants of the Liliaceae family

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

I. Medicinal plants of the Caryophyllaceae and Boraginaceae families

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

J. The Boraginaceae or borage family

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

K. Medicinal plants of the Ranunculaceae and Papaveraceae families

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

L. The Papaveraceae or poppy family

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

M. Medicinal plants of the Malvaceae and other families

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

N. The Cucurbitaceae or cucumber family

It contains a large number of edible crops such as the cucumbers, melons and pumpkins. Two important medicinal plants in this family include the squirting cucumber (Ecballium elaterium) and the pumpkin (Cucurbita maxima). Another family, called the Verbenaceae or verbena family contains three important medicinal plants; vervain (Verbena officinalis), chaste tree (Vitex agnus-castus) and the cultivated lantana (Lantana camara) An important and common medicinal plant of the Scrophularia or figwort family is the snapdragon (Antirrhinum majus). It is a native of West Mediterranean and grows on rocky grounds and old walls. It flowers from January till October. (Gupta,2008)

1.19 Uses of medicinal plants

1. Herbs such as black pepper, cinnamon, aloe, red clover,bayberry are used to heal wounds,sores and boils.

2. Some herbs also having antibiotic properties.Turmeric is useful in inhibiting the growthof germs,harmful micobes and bacteria.Turmeric is widely used as a home remedy to heal cut and wounds.

3. To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as chirayta,sandalwood, and sunflower are recommended by traditional Indian medicine practitioners.

4. Sandalwood and cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.

5. Indian sages were known to have remedies from plants which act against poisons from animals and snack bites.

6. Ginger and cloves are used in certain cough syrups .They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs ,trachea and bronchi.

7. Herbal medicine practitioners recommended calmative herbs, which provide a smoothing effect to the body. They are often used as sedatives.

8. Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like cayenne.

9. A wide variety of herbs including Giloe ,Golden seal, Aloe are used as tonics .They can also be nutritive and rejuvenate a healthy as well as diseased individual.

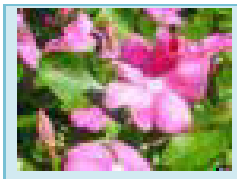
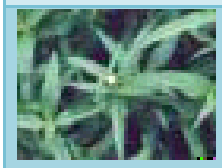
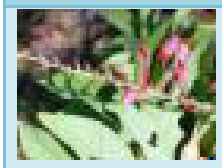
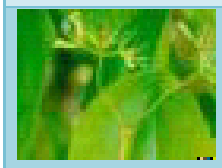
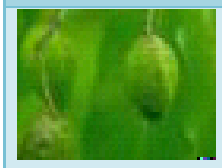
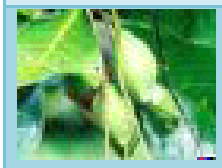
10. Certain aromatic plants such as Aloe, Golden seal ,Barberry are used as mild tonics.

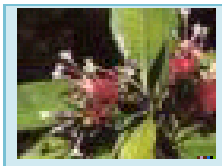
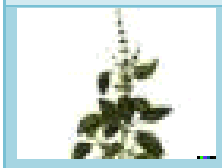
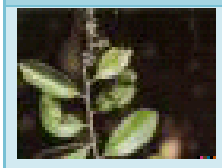
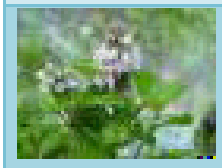
11. Many herbs are used as blood purifier to alter or change a long lasting condition by eliminating the metabolic toxins.

12. Some medicinal plants have disinfectant property ,which destroys disease causing germs.

13. Honey, turmeric ,mashmallow and liquorices can effectively treat a fresh cut and wound. They are termed as vulnerary herbs. (Zahid,2016)

Table 2 : Example of some important herbs

	Sada Bahar Family:Apocyanaceac	Whole plant	Leaukamia,Anti-spasmodic
	Vringraj Family: Compositae	Whole Powder	Anti-inflammatory,Digestive
	Rakta chitrak Family:Plumbaginaceac	Root	Inflammation,Cough
	Kochila Family:Loganiaceac	Seed	Nervous,Paralysis,Wound
	Harida Family: Combretaceac	powder	Ulcer,Leprosy,Cough
	Bahada	Seed,Bark,Fruit	Insomnia,Vomiting
	Neem	Rhizome	Urinary,Sedative,Hypertensive

	Sarpa Ghandha Family:Apocynaceac	Root	Hypertension
	Satavari Family: Liliaceac	Root	Fatigue,Cough
	Senna Family: Liliaceac	Dry tubers	Tonic,Aphrodisiac
	Tulsi Family:Lamiaceac	Leaves	Cough,cold,Bronchitis
	Vai vidanka Family: Myrsinaceac	Leaves , fruit	Skin disease,Snike bite
	Pippermint perennial Family: Lamiaceac	Leaves, Flower	Digestive,Pain killer
	Henna,Mehedi Family: Lytharaceac	Leaf,Flower	Anti-inflammatory
	Gritkumari Family: Liliaceac	Leaves	Laxative,Wound,Skin burn

1.20 Antioxidants

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

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

1.21 Classification of Antioxidants

Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time (Touriño et al. 2008).

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

1.21.1 Natural Antioxidants

Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and non-enzymatic antioxidants.

1.21.1.1 Enzymatic Antioxidants

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

1.21.1.2 Primary Antioxidants

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

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

1.21.2 Secondary Antioxidant

Secondary antioxidant includes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is required to recycle the reduced glutathione (GSH) using secondary enzyme GR and NADPH (6.4).

GSSG + NADPH ¾G¾R ® NADP + 2GSH (6.4)

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

1.21.2.1 Non enzymatic Antioxidants

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

1.21.2.2 Minerals

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

1.21.2.3 Iron (Fe)

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

1.21.2.4 Magnesium (Mg)

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

1.21.2.5 Selenium (Se)

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

1.22 Garcenia genus

Garcinia is a plant genus of the family Clusiaceae native to Asia, Australia, tropical and southern Africa, and Polynesia. The number of species is highly disputed, with various sources recognizing between 50 and about 300. Commonly, the plants in this genus are called saptrees, mangosteens (which may also refer specifically to the purple mangosteen, G. mangostana), garcinias or, ambiguously, "monkey fruit".

Scientific classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Rosids

Order: Malpighiales

Family:Clusiaceae

Tribe: Garcinieae

Genus:Garcinia

Synonyms

Brindonia Thouars

Cambogia L.

Clusianthemum Vieill.

Mangostana Gaertn.

Oxycarpus Lour.

Pentaphalangium Warb.

Rheedia L.

Septogarcinia Kosterm.

Tripetalum K.Schum.

Tsimatimia Jum. & H.Perrier

Verticillaria Ruiz & Pav.

Xanthochymus Roxb.

Many species are threatened by habitat destruction, and at least *G. cadelliana* from South Andaman Island is almost or even completely extinct already.The fruits are a food source for several animals, such as the archduke butterflies (Lexias) of tropical eastern Asia which relish the sap of overripe mangosteens.

Description

Garcinia species are evergreen trees and shrubs, dioecious and in several cases apomictic. The fruit is a berry with fleshy endocarp,[2] which in several species is delicious.

Uses

Fruit of the purple mangosteen (Garcinia mangostana), together with its cross section; note the white edible endocarp.

The fruit of most species of Garcinia are eaten locally; some species' fruits are highly esteemed in one region, but unknown just a few hundred kilometres away. The best-known species is the purple mangosteen (G. mangostana), which is now cultivated throughout Southeast Asia and other tropical countries, having become established in the late 20th century. Less well-known, but still of international importance, are kandis (G. forbesii) with small round red fruits with subacid taste and melting flesh, the lemon drop mangosteen (G. intermedia) with yellow fruit that look like a wrinkled lemon, and the thin-skinned orange button mangosteen (G. prainiana).

In addition, mangosteen rind (exocarp) extract is used as a spice. It figures prominently in Kodava culture, and G. multiflora is used to flavour and colour the famous bún riêu soup of Vietnam, where this plant is known as hạt điều màu. Garcinia gummi-gutta yields a spice widely used in South Asia, in particular in Kerala, where it is called kodumpulli.

Most species in Garcinia are known for their gum resin, brownish-yellow from xanthonoids such as mangostin, and used as purgative or cathartic.

Extracts of the exocarp of certain species – typically *G. gummi-gutta*, but also purple mangosteen – are often contained in appetite suppressants such as Hydroxycut, Leptoprin or XanGo. But their effectiveness at normal consumption levels is unproven, while at least one case of severe acidosis caused by long-term consumption of such products has been documented.Furthermore, they may contain significant amounts of hydroxycitric acid, which is somewhat toxic and might even destroy the testicles after prolonged use.Bitter kola (G. kola) seeds are used in folk medicine.[citation needed] G. mannii is popular as a chew stick in western Africa, freshening the breath and cleaning the teeth.

G. subelliptica, called fukugi in Japanese, is the floral emblem of Mobuto and Tarama on Okinawa. The Malaysian town of Beruas – often spelled "Bruas" – derives its name from the seashore mangosteen (*G. hombroniana*), known locally as pokok bruas.

1.23 Morphological characteristics of garcenia genus

The genus Garcinia (Family: Clusiaceae) consists of over 200 species distributed in the tropics of the world chiefly in Asia, Africa, and Polynesia. They are evergreen polygamous trees, shrubs, and herbs. About 35 species are reported to exist in India, many of which are endemic and economically important with immense medicinal properties.In India, species of Garcinia grow extensively in semiwild state, in the Konkan region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, and evergreen forests of Assam, Khasi, Jantia hills, Nagaland, West Bengal, and Gujarat. In Malabar and Konkan regions of Southern India, they are used in garnishing curries and also as a replacement for tamarind. In North Eastern India, the sundried slices of the fruits are used for culinary purposes and as folk medicine.

Some species like *Garcinia cambogia*, *G. indica*, and *G. cowa* are cultivated in certain parts of India. *G. pedunculata*, *G. kydia*, *G. cowa,* and *G. lanceaefolia* are the most important species in north eastern parts of India. Many species of Garcinia have fruit with edible arils and are eaten locally. The best-known species is the mangosteen *(G. mangostana*), which is now cultivated throughout Southeast Asia and other tropical countries. The seeds of *G. indica* fruits yield valuable edible fat known as kokum butter. The fruits of Garcinia are a food source for several animals. Most species in Garcinia are known for their gum resin which is used as purgative or cathartic.

In this study, we tried to characterize the morphological features of the species collected from Western Ghats and Himalayan Foot hills. Nine species studied here are the most common species of the two ecosystems, but the awareness about the crop or its medicinal value is very less. Most Garcinia species occur only as natural populations and are known only locally. Many Garcinia species have edible arils and are eaten locally. Some species fruits are highly esteemed in one region but are unknown just a few hundred kilometers away. Perhaps, trees are cut due to lack of awareness and popularization of importance of Garcinia will help in conserving the local populations of this genus. Here it is worth to mention that the climatic parameters of both ecosystems are almost the same specially the altitude and rainfall pattern. The altitude varies from 100 to 600 MLS, while the annual rainfall varies from 1500 to 4500. (Utpala,2014)

1.24 *Gercenia cowa*

The Genus Garcinia, belonging to the Family Clusiaceae have been widely investigated in terms of their bioactive ingredients. The plants are small to medium sized trees, which grow up to 30 m in height and are widely distributed in the tropical regions of the world (Kijjoa and Vieira, 2009). This genus has various biological activities such as antioxidant (Muharni et al., 2009 and Dachriyanus et al., 2003), cytotoxic (Wahyuni et al., 2009) and antimicrobial activities (Dachriyanus et al., 2004). *Garcinia cowa Roxb* known as asam kandis in West Sumatera It is widely distributed throughout Indonesia and the Malay peninsula. The fruits are edible with a sour taste and used as spices in Indonesia especially in Minang tribes. (Dachriyanus et al., 2003)

Fig 5: *Garcenia cowa* whole plant

1.25 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

1.26 Taxonomical Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Clusiaceae

Genus: Garcinia

1.27 Distribution

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

1.28 Parts Used

Leaves, barks , flower, stem and fruits.

1.28.1 Fruits

Cowa is a lesser known edible fruit found in the states of East India (Assam, Mizoram, Bengal, Bihar and Orissa). It is also found in the Andaman and Nicobar Islands. It occurs wild frequently in evergreen and semi evergreen forests or along streams in deep valleys.

Besides India, it is also reported to grow in South China, Bangladesh, Malaysia, Laos, Cambodia and Vietnam.In Mizoram, cow is cultivated as a subsidiary crop in Citrus, banana and arecanut orchards.

Fig 6: *Garcenia cowa* fruit

Description

Trees 8-12 m tall, 15-20 cm in diam; bark dark brown; branches many, borne toward top of trunk, horizontal but usually distally pendulous, slender; twigs dark brown, striate.

Fruit opaquely yellow-brown, ovoid-globose, oblique, 5-6 × 4-5 cm in diam., 4-8-sulcate, usually apiculate, pinkish red, looking similar to tomato. Seeds 2-4, narrow, fusiform, slightly curved, ca. 2.5 cm, rough.Petiole 0.8-1.5(-2) cm; leaf blade lanceolate or oblong-lanceolate, 6-14 × 2-5 cm, papery, midvein raised abaxially, impressed adaxially; secondary veins 12-18 pairs, near margin joining together; tertiary veins conspicuous on both surfaces, base cuneate, sometimes slightly decurrent, margin cartilaginous, involute, apex acuminate or long acuminate, rarely acute or obtuse.

Utilization

The fruits are edible. In spite of their being slightly sour in taste, these are fondly eaten by local people especially in Mizoram. The fruits are also made into jam and preserve. In East India, the sun dried slices of this fruit are used to treat dysentery. The young leaves are cooked and eaten as a vegetable. The bark is used for dying clothes yellow.

Flower

Male flowers 3-8, terminal or axillary, in an umbel; umbel shortly pedunculate or rarely sessile, 4-bracteate at base; bracts subulate; pedicels 4-8 mm, slender; petals yellow, ca. 2 × as long as sepals; stamen fascicles 4, connate, forming a central capitate 4-sided mass of 40-50 anthers; filaments ± absent, at most short, anthers 4-celled, cells longitudinally dehiscent; pistillode absent; female flowers usually solitary, axillary, larger than male; pedicels robust, 2-3 mm; staminodes united in lower half and enveloping ovary base; filaments long or short, usually shorter than ovary; ovary ovoid, 4-8-loculed; stigma radiately 4-8-lobed, papillate, 6-7 mm high.

The bark, latex and root have been used as an antipyretic agent (Mahabusarakam, et al, 2005 and Pathong et al,2009) while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant (Pathong et al., 2009). Some pharmacological properties such as antitumor- promoting (Mukarami et al., 1995), inhibition of human low- density lipoprotein peroxidation and anti-platelet activities have been reported on the crude extract of leaves (Jantan et al., 2011).

Stem

The chemical composition and biological activities of various parts of *G. cowa* have been investigated. Previous investigation on the fresh leaves, fruits and dried rinds of *G. cowa* has been investigated and found that (-)-hydroxycitric acid and its lactone constitute the major constituents (Jena et al., 2002). Previously, we reported the isolation of [2E,6E,10E]-(+)-4 -hydroxy-3-methyl-5 -(3,7,11,15- tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one (1), 2- (3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4- (1,1-dimethyl- 2-propenyl)-9H- xanthen-9-one (2) and rubraxanthone (3) from the stem bark of this plant. (Wahyuni et al., 2004)

Leaves

In continuation of our study on*Garciniacowa* (Wahyuni et al., 2004), cytotoxic properties of isolated compounds from the leaves of *Garcinia cowa* against cancer. ((Dachriyanus et al., 2003)

Fig 7: *Garcenia cowa* leaves

1.29 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,

1.30 Species

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

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

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

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

1.31 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)

Wood

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

Medicinal Uses

Dried fruits are shipped from Singapore to Calcutta and to China for medicinal use. The sliced and dried rind is powdered and administered to overcome dysentery. (Ghani,1998)

1.32 Classes of Compounds Isolated from *Garcinia cowa*

1.32.1 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.

1.32.2 Flavonoids

Twelve flavonoids (compounds 2-13 in Table 1) were isolated from *G. cowa* with garccowasides A (6), B (7) and C (8) being first reported as new compounds [18]. Of these compounds, only morelloflavone (11) and morelloflavone-7 -O-glucoside (13) showed strong antioxidant activities.

1.32.3 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.. 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 pneumoni*a (MTCC-618) with 15 and 15 mm of clear zones respectively as determined by the agar diffusion method.

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*

1.32.4 Xanthones

Xanthones, with two aromatic rings linked via carbonyl and ether linkages, are the major components of the Garcinia genus. They are commonly found in several parts of *G. cowa*, especially in the stem, fruit and latex. Thirty six xanthones (46% of the total isolated compounds) have been isolated and nineteen of them were first isolated from *G. cowa*. They are cowagarcinone, cowaxanthone, cowanol, cowanin, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3- methyl-2-butenyl)xanthone, norcowanin, cowagarcinones A (49), B (50), E (51) and D (52) from the latex [15, 30]; cowaxanthones B (34), C (39), D (42) and E (44) from the fruit [20]; 7- O-methylgarcinone E (36), 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone (59), 4-(1,1-dimethyl-prop-2-enyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthen-9(9H)-one (61) and 1,5-dihydroxy-3methoxy-6′,6′-dimethyl-2H-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone (62) from the stem [18, 33]; and cowaxanthone F (55) from the twig. Most of these xanthones showed interesting biological activity.Cowaxanthone, cowanin, norcowanin and 3,6-di-O-methyl- - mangostin exhibited significant cytotoxicity against the NCI-H187 cell line with IC50 values ranging between 3.87-8.58 µg/mL, and moderately inhibited KB and MCF-7 cancer cell lines

1.32.5 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.

1.32.6 Anti-inflammatory activity

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 IC50 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. (Academic library,2014)

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 IC50 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.

1.32.7 Miscellaneous Compounds

Ten (13% of the total isolated compounds) of the miscellaneous class of compounds have been isolated, including a new discovery: (2E,6E,10E)-(+)-4 -hydroxy-3-methyl-5 -(3,7,11,15- tetramethyl-hexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one (68).None of the isolated compounds from this class were tested for their biological activities. (Academic library ,2014)

1.33 Aim of this experiment

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases.

The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity..

Garcinia cowa is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on this plant. The principal aim of the present study was to investigate the scientific basis of the traditional uses of the plant. The methanolic extract of to evaluate their in- vitro pharmacological activities (like antioxidant, antimicrobial).

Chapter 2
Literature Review

2.1 Literature Review on *Garcinia cowa*

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

2.1.1 Antioxidant and Antiplatelet Aggregation Properties of the BarkExtracts of *Garcinia pedunculata* and *Garcinia cowa*

The bark extract of *Garcinia pedunculata* and *Garcinia cowa*, which is abundant in the Northeastern regions of India, were screened for their antioxidant and in vitro antiplatelet aggregating activities. By β-carotene linoleate model for antioxidant assay, acetone extract of *G. pedunculata* and hexane extracts of *G. cowa* exhibited higher antioxidant activity (86.47 and 66.94 % respectively, at 25 ppm) than other extracts. Similar pattern was observed for superoxide radical scavenging method for antioxidant assay. The ethyl acetate extract of G. pedunculata and hexane extract of G. cowa exhibited higher antiplatelet aggregation capacity towards ADP induced platelet aggregation (IC50 0.16 and 0.43 ug, respectively) than other extracts. (0mali et al, 2008)

2.1.2 Cowaxanthone F and Other Anti-inflammatory and AntioxidantCompounds from *Garcinia cowa*

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

2.1.3 Cytotoxic Compounds from the Leaves of *Garcinia Cowa*

The aim of this study was to isolate compounds from the leaves of methanol extract of *Garcinia cowa* and to evaluated their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines. The dichloromethane fraction was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature these compounds were elucidated as methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate (1), garcinisidone-A (2) and methyl 4,6dihydroxy-2-(4-methoxy-5-(3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4-dienyloxy)-3-(3-methylbut-2-enyl)benzoate (3). Compound 1, 2 and 3 had IC50 value of 21.0 ± 10.2 µM, 21.2 ±8.4 µM and 17.2 ± 6.2µM against MCF-7, while only compound (2) was found to be in active against H-460 with IC50 value of 18.1 ± 6.7 µM. Conclusion: The results indicate that *G. cowa* leaves could be important sources of natural cytotoxic compounds and only compound (2) had activity against H-460 cell lines.(Chowdhuary et al ,2013)

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

Organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa (G. cowa)* were determined by high-performance liquid chromatography. Fresh leaves, fruits, and dried rinds were extracted with water at 120 degrees C for 20-30 min under 15 lbs/in(2) pressure. Also, dried rinds were extracted with solvents (acetone and methanol) using a Soxhlet extractor at 60 degrees C for 8 h each. The samples were injected to HPLC under gradient elution with 0.01 M phosphoric acid and methanol with a flow rate of 0.7 mL/min using UV detection at 210 nm. The major organic acid was found to be (-)-hydroxycitric acid present in leaves, fruits, and rinds to the extent of 1.7, 2.3, and 12.7%, respectively. (-)-Hydroxycitric acid lactone, and oxalic and citric acids are present in leaves, fruits, and rinds in minor quantities. This is the first report on the composition of organic acids from *G. cowa*. (Utpala ,2014)

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

Garcinia is a plant under the family of Clusiaceae that is commonly used as a flavouring agent. Various phytochemicals including flavonoids and organic acid have been identified in this plant. Among all types of organic acids, hydroxycitric acid or more specifically (−)-hydroxycitric acid has been identified as a potential supplement for weight management and as antiobesity agent. Various in vivo studies have contributed to the understanding of uptake. Besides, it also helps to enhance fat oxidation while reducing de novo lipogenesis. However, results from clinical studies showed both negative and positive anti-obesity effects of Garcinia/hydroxycitric acid. This review was prepared to summarize the update of chemical constituents, significance of in vivo/clinical anti-obesity effects, and the importance of the current market potential of Garcinia/hydroxycitric acid.

1. Garcinia has been used for centuries in Asian countries for culinary purposes as a condiment and flavoring agent in place of tamarind or lemon and to make meals more filling.Besides its use as a flavouring agent, the dried rind of *G. cambogia* combined with salt and other organic acids can help to lower the pH and thus provides a bacteriostatic effect in curing fish. *G. cambogia* contains large amounts of hydroxycitric acid (HCA). Similar to *G. cambogia,G. atroviridis* and *G. indica* also contain significant HCA content and are sometimes used interchangeably with *G. cambogia* in food preparation.In spite of the vastly reported prominent role of HCA in inducing satiety, reduced energy intake and weight gain, and improved blood parameters and substrate oxidation, controversial results regarding its efficacy and safety as an anti-obesity dietary supplement had also been reported. Evidence from the in vitro, in vivo, and clinical trials on the safety of Garcinia/HCA as a dietary supplement for treating obesity had been extensively reviewed. However, the efficacy of Garcinia/HCA remains the subject of debate. Despite the previously stated issues, on conclusive evidence for HCA'.from a very broad range of reports, rigorous clinical trials, systematic reviews, and meta- analyses on the efficacy and potential of Garcinia/HCA as an anti-obesity dietary supplement.

2. Garcinia extract has been used in the traditional Ayurvedic medical system .A decoction of *G. cambogia* is given as purgative in the treatment of intestinal worms and other parasites, for bilious digestive conditions, for dysentery, rheumatism, and in the treatment of tumours. Less commonly, extracts are employed as cardiotonics to treat angina. In veterinary medicine, it is used as a rinse for diseases of the mouth in cattle. The fruit rind is used in rickets and enlargement of spleen and to heal bone fractures. In Southeast Asian folkloric medicine, a decoction of *G. atroviridis* (leaves and roots) is sometimes used for the treatment of cough, dandruff, earache, stomach pains associated with pregnancy, and throat irritation. The dried fruit of *G. atroviridis* is used for improving blood circulation, for the treatment of coughs, as a laxative, and as a expectorant. The fruit is used in a lotion with vinegar to rub over the abdomen of women after confinement. Fruit of *G. indica* is antiscorbutic, cholagogue, cooling, antibilious, emollient, and demulcent. The anthelmintic properties of the fruit of *G. indica* contributed to its use in haemorrhoids, dysentery, tumor, pains, and heart complaints. Bilious affected sites are treated with syrup from the fruit juice.(Okunji et al.,2016).

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

The methanol extracts of 32 plant parts of 19 species of the genus Garcinia (Guttiferae) were collected from rainforests of the Malaysian Peninsula and the island of Sumatra, Indonesia, for evaluation of their in vitro cytotoxic and nitric oxide inhibitory activities. An end-point MTT cell viability assay was used to determine the 50% inhibitory concentration (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 cow*a (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. malaccensi*s (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. (Jantan et al,2011)

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

The crude hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* were studied for their antibacterial activity against some foodborne pathogens and spoilage bacteria such as *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentrations (MICs) of the extracts determined by the agar dilution method were ranging from 15 to 500 µg/ml and 300 to 1250 µg/ml for *G. cowa* and *G. pedunculata*, respectively. However, the hexane and chloroform extracts from the fruit rinds of *G. cowa* exhibited marked inhibitory effect against all the test organisms and were more effective than that of *G. pedunculata* extracts. The antibacterial activity of all the extracts was more pronounced against the tested Gram-positive bacteria than the tested Gram-negative bacterium. Furthermore, this study is the first report on the in vitro antibacterial activity of extracts from the fruit rinds of *G. cowa* and *G. pedunculata*. (Haque et al, 2012)

2.1.8 Xanthones from the Leaves of *Garcinia Cowa* Induce Cell Cycle Arrest

Two new xanthones, cowaxanthones G (**1**) and H (**2**), and 23 known analogues were isolated from an acetone extract of the leaves of *Garcinia cowa*. The isolated compounds were evaluated for cytotoxicity against three cancer cell lines and immortalized HL7702 normal liver cells, whereby compounds **1**, **5**, **8**, and **15**−**17** exhibited significant cytotoxicity. Cell cycle analysis using flow cytometry showed that **5** induced cell cycle arrest at the S phase in a dose-dependent manner, **1** and **16** at the G2/M phase, and **17** at the G1 phase, while **16** and **17** induced apoptosis. Moreover, autophagy analysis by GFP-LC3 puncta formation and western blotting suggested that **17** induced autophagy. Taken together, our results suggest that these xanthones possess anticancer activities targeting cell cycle, apoptosis, and autophagy signaling pathways.(Kumar P,2014)

2.1.9 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 Staphylococcusaureus)* and one Gram-negative bacterium (*Escherichia coli*). Both extracts significantly inhibited bacterial, but not E. coli (IC_{50}s 250-500 ·growth of the Gram-positive bacteria (IC_{50}s 15-30). 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)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. The structural types, chemical structures and biological activities of the natural products isolated from different parts of *G. cowa(*Maejo Int. J. Sci. Techol. 2013).

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2.1.10 Antifever Activity 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 antifever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant . The chemical composition and biological activities of various parts of G. cowa have been investigated. The major compounds found were xanthones and phloroglucinols. However, minor compounds, including depsidones, terpenoids, steroids and flavonoids, were also observed. Currently, 78 compounds have been isolated from the twig , stem , fruit and latex . This review mainly focuses on the chemical structures and biological activities of the phytochemicals isolated from G. cowa and covers the literature up to April 2012. (Dachriyanus et al,2003)

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Chapter 3
Methodology

3.1 Preparation of Plant Extract for Experiments

3.1.1 Collection & Preparation of Plant Material

Garcinia cowa plant was collected in the month of June, 2014 from Comilla,Barishal during rainy season when weeds were in their maximum densities. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves was 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* stem

At first the leaves were thoroughly washed with tap water .The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 500C 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 leaf was 158 gm which was measured using electronic balance and it was found to be 158 gm.

3.1.4 Extraction of the Dried Powdered Sample

The fine powder of *Garcinia cowa* stem was dissolved in 1000 ml methanol 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 Solvent Evaporation

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

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.

3.2 Principle of a Rotary Evaporator

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

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

Figure 8 : Drying of extract using rotary evaporator

3.3 Theory of Phytochemical Screening

3.3.1 Materials (Reagents and Tools) Used

Table 3 : Reagents Used in Phytochemical Screening

Reagents & Tools	
Molishch's reagents (10% naphthol in alcohol) - for carbohydrate test.	Conc. Hydroclric acid – for flavanoid test.
Dilute sulphuric acid and NaOH solution- for glycoside test.	Conc. Sulphuric acid- for steroid test.
Aqueous sodium hydroxide solution- for glycoside test.	$FeCl_3$ (5%) - for tannin test.
Fehling's solution- for glycoside test.	Solvents – alcohol, chloroform and distilled water.

10% Ammonia solution- for anthraquinone glycoside test.	Test tube
Mayer's reagent (potassiomercuric iodide solution)	Watch glass
Wagner's reagent (solution of I in KI)	Holder
Hager's reagent (Saturated solution of picric acid).	Burner
Dragendroff's reagent (Bismuth sub nitrate and acetic acid solution)- All for alkaloid tests.	

3.3.1 Test Compounds

Dichloromethane extract of *Gaecinia cowa* stem

3.3.2 Preparation of Sample Solution

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

3.3.3 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. H2SO4 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. **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 H2SO4. 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.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

iii. 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) **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.

iv. 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

Forrmation 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.

v. 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.

vi. Test for flavanoids

A few drops of conc. HClwas added to a small amount of an extract. Immediate development of a red color 7indicated the presence of flavonoid.

vii. 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.

viii. Test for tannins

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

3.3.4 Assessment of In Vitro Pharmacological Property

3.4 Determination of Antioxidant property

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.

Table 4: Reagents used in DPPH free redical seavenging assay

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

3.4.1 DPPH Solution

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

3.4.2 Preparation of Standard/ Extract solution

0.025 gm ascorbic acid or extract was taken and dissolved into 5 ml of Absolute ethanol. The concentration of the solution was 5mg/ml of ascorbic acid/ extract.

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

Table 5 : Amount of standard or extract solution

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

3.5 Procedure

i) 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.

ii) Each test tube contains 1ml of each concentration and is properly marked.

iii) 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).

iv) Incubate the mixture in room temperature for 30 minutes in a dark place.

v) Then the absorbance is measured at 517 nm against dilute extract solution in the solvent

Calculation

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.

3.6 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).

3.6.1 Materials

3.6.2 Microorganisms

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

3.6.3 Test Organisms

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

Table 6: List of bacteria

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

3.6.4 Culture Media and Chemicals

I. Nutrient agar media
II. Ethanol
III. Chloroform

3.6.5 Equipments

Table 7 : Equipments

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

3.6.6 Test Materials

The Dichloromethane extract of *G.cowa* stem were tested against gram-positive and gram-negative bacteria.

3.7 Methods

3.7.1 Culture Preparation

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

3.7.2 Composition of culture media

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

3.7.3 Composition of Nutrient agar media (1000 ml)

Table 8 : Composition of Nutrient agar media

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

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25^0C) was adjusted at 7.2 ± 0.1 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15 lbs pressure/sq. inch at 121˚C for 20 min. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study

3.7.4 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. ere also sterilized.

3.7.5 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.

3.7.8 Preparation of the Test Plates

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

3.8 Preparation of Discs

3.8.1 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.

3.8.2 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.

3.8.3 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.

3.8.4 Placement of Disc and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 40˚C for about 24 h. Finally the plates were **3.2.2. 3.8.5 3.8.5**

3.8.5 Determination of Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a

Chapter 4

Results and discussion

4.1: Phytochemical screening of Dichloromethane Extract of *Garcinia cowa* stem

Table 9 : Phytochemical screening test result

Test	Result
Carbohydrate	-
Glycosided	+
Alkaloid	-
Saponina	-
Flavanoids	+
Steroids	+
Tannins	-

4.2 DPPH test of Dichloromethane Extract of *Garcinia cowa* stem

4.2.1 Preparation of DPPH scavenging activity curve

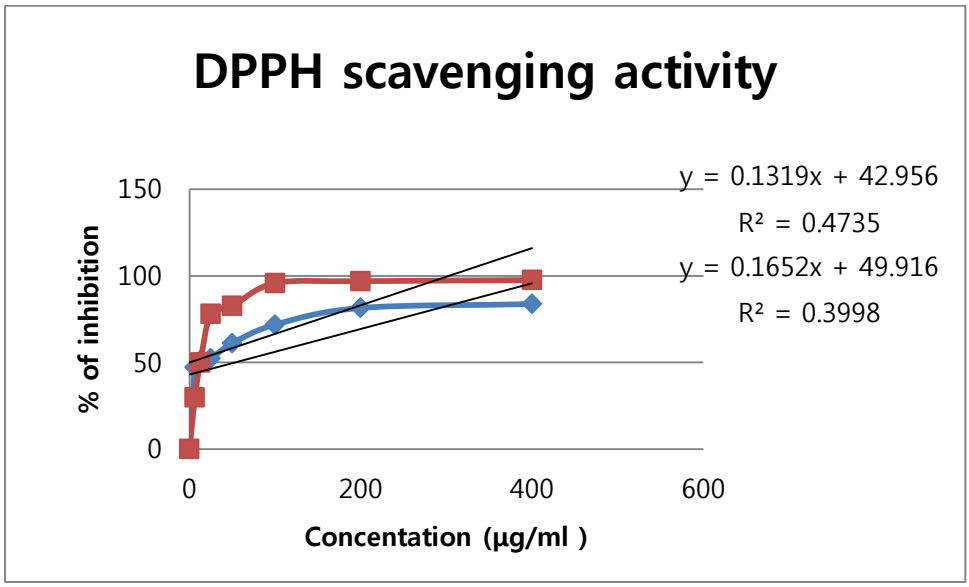

Figure 9 : DPPH scavenging activity

4.2.2 Result of DPPH test

Table 10 : DPPH scavenging activity

Dichloromethane Extract of *G.cowa stem* /AscorbicAcid	**IC_{50}Value(µg/ml)**	**Regression Line**	**R^2Line**
Dichloromethane Extract of *G.cowa* stem	53.404	Y=0.131x+ 42.95	R^2=0.473
AscorbicAcid	1.8214	Y=0.165X+49.91	R^2=0.399

4.3 Total phenol test:

4.3.1 Preperation of standard curve for Gallic acid

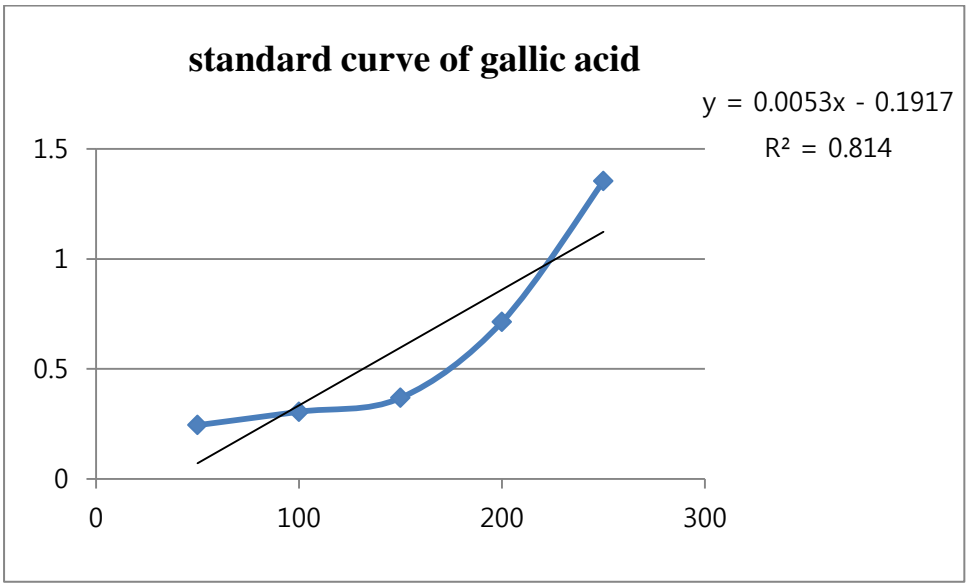

Figure 10 : standard curve for Gallic acid

4.3.2 : Result of total phenol content

Table 11 : Result of total phenol content

Serial no.	Absorbance of Dichloromethane extract of *G.cowa* stem	Standard equation	Value of x	Mean	Standard deviation	Total Phenol Content
1	0.673		172.8			
2	0.653	0.0053x-0.1917	168.8	173.93	5.78	173.93 ± 5.78
3	0.71		180.2			

4.4. Antimicrobial Screening of Dichloromethane Extract of *Garcinia cowa* stem

Table 12 : result of Antimicrobial Screening of Dichloromethane Extract of *Garcinia cowa* stem

Name of Bacteria	Dichloromethane Extract of *G.cowa* Stem(300 µg/disc)	Dichloromethane Extract of *G.cowa* Stem (600µg/disc)	Zone of Inhibition of Zentamycin (30µg/disc)
Sarcina lutea	8 mm	16mm	30 mm
Bacillus megaterium	15 mm	16 mm	30 mm
Bacillus subtilis	8 mm	7 mm	30 mm
Salmonella paratyphi	8 mm	7 mm	30 mm
Salmonella typhi	6 mm	8 mm	30 mm
Vibrio parahemolyticus	7 mm	6 mm	30 mm
Staphylococcus aureus	8 mm	6 mm	35 mm
E.coli	7 mm	8 mm	30 mm
Shigella dysenteriae	12 mm	6 mm	35 mm
Pseudomonas aureus	15 mm	12 mm	30 mm

Discussion:

Herbal medicines have received high interest as a substitute to clinical treatment, and the demand for herbal remedies has currently increased rapidly. The increase in the number of herbal users as opposed to the insufficiency of scientific evidences on its safety has raised concerns regarding its detrimental effects and related concerns apply to the *Garcinia cowa* in this study. *Garcinia cowa* is a medicinal plant enriched with various chemical constituents having different medicinal activities. The study has shown the antioxidant and antimicrobial activities. It is used as a medicine for the treatment of various diseases. Dried fruits are shipped from Singapore to Calcutta and to China for medicinal use. The sliced and dried rind is powdered and administered to overcome dysentery. Made into an ointment, it is applied on eczema and other skin disorders. The rind decoction is taken to relieve diarrhea and cystitis, gonorrhea and gleet and is applied externally as an astringent lotion. A portion of the rind is steeped in water overnight and the infusion given as a remedy for chronic diarrhea in adults and children. Filipinos employ a decoction of the leaves and bark as a febrifuge and to treat thrush, diarrhea, dysentery and urinary disorders. In Malaya, an infusion of the leaves, combined with unripe banana and a little benzoin is applied to the wound of circumcision.Due to its huge therapeutic use by the tribal I get interested to do experiment on this plant. The therapeutic value of medicinal plants lies in the various chemical constituents in it. Phytochemical screening showed that the dichloromethane extract of *Garcinia cowa* stem was rich in phytochemical constituents. Such as- Flavonoid, Steroid, Carbohydrates and Tannin compounds. Thus further research is needed to work out the active medicinal compounds present in this extract; used for the treatment of various types of diseases. In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect. In the present study the dichloromethane extract of *Garcinia cowa* stem showed the activity against *Bacillus sereus, Salmonella paratyphi, Vibrio parahemolyticus, E.coli* and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The antioxidant activity was measured by Phytochemical Screening, DPPH and Total Phenol tests. IC_{50} values of DPPH tests were 53.404 µg/ml for dichloromethane extract of *G.cowa* stem. The Total Phenol contents 173.93 ± 5.78 mg/g equivalent to Gallic Acid for Dichloromethane extract of *Garcinia cowa* stem. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. It becomes difficult to describe the all properties selectively to any one group of constituents without further studies, which are beyond the

scope of this paper. Thus, further extensive investigations are necessary to find out the active principles present in these plants. The antimicrobial activity of the dichloromethane extract of *Garcinia cowa* stem was tested against ten microorganisms. The highest antimicrobial activity was shown against *Sarcina Lutea* and *Pseudomonas aureus*.The diameter of the zone of inhibition of *Sarcina Lutea* was 16 mm (600 µg/disc) compared to the 30 mm of diameter of zone of inhibition of the standard zentamycin 30 µg/disc and Pseudomonas Aureus was 15 mm (300 µg/disc). It shows the moderate activity against *Bacillus sereus, Vibrio parahemolyticus* and *E.coli* I n case of 300 µg/disc,. It showed no activity *against Bacillus megaterium, Staphylococcus aureus, Shigella dysenteriae , Pseudomonas aureus*. So the dichloromethane extract of *Garcinia cowa* stem 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.

Chapter 5

Conclusion

Conclusion

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

Chapter 6

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