# PHYTOCHEMICAL SCREENING, ANTIOXIDANT & ANTIMICROBIAL INVESTIGATIONS OF PETROLEUM ETHER EXTRACT OF *Garcinia cowa* LEAVES

***A Dissertation Submitted to the Department of Pharmacy, East West University in the Partial Fulfillment of the Requirements for The Degree of Bachelor of Pharmacy***


**Submitted By**

Sabrina Islam

2014-1-70-011

**Department of Pharmacy**

**East West University**

# Declaration by the Research Candidate

I, **Sabrina Islam**, ID: 2014-1-70-011, hereby declare that the dissertation entitled "**Phytochemical Screening, Antioxidant and Antimicrobial Investigations of Pet Ether Extract of *Garcinia cowa* Leaves**" submitted by me to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy is a record of research work under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

_____

Sabrina Islam

ID: 2014-1-70-011

Department of Pharmacy

East West University

# Certificate by the Supervisor

This is to certify that the thesis entitled "**Phytochemical Screening, Antioxidant and Antimicrobial Investigations of Pet Ether Extract of *Garcinia cowa* Leaves**" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by **Sabrina Islam**, ID: 2014-1-70-011 in 2017 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me.

_____

Nazia Hoque

Assistant Professor

Department of Pharmacy

East West University

# Certificate by the Chairperson

This is to certify that the thesis entitled "**Phytochemical Screening, Antioxidant and Antimicrobial Investigations of Pet Ether Extract of *Garcinia cowa* Leaves**" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by **Sabrina Islam**, ID: 2014-1-70-011 in 2017.

_____

Dr. Chowdhury Faiz Hossain

Professor and Chairperson

Department of Pharmacy

East West University

# Acknowledgements

All praise is for Almighty for all the bounties granted to me and only with His guidance and help this achievement has become possible.

It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected teacher and supervisor **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, for her expert supervision, constructive criticism, valuable advice, optimistic counseling, constant support and continuous backup and encouragement throughout every phase of the project as well as to prepare this dissertation.

I would also like to put forward my most sincere regards and profound gratitude to **Dr. Chowdhury Faiz Hossain**, Professor and Chairperson, Department of Pharmacy, East West University, for giving me the opportunity to conduct such an interesting project and for facilitating a smooth conduction of my study.

I would also like to extend my thanks to all the research students in the lab, lab officers and other staffs of the Department of Pharmacy for their help and assistance, friendly behavior and earnest co-operation which enabled me to work in a very congenial and comfortable ambience.

I owe special thanks to my fellow research group members for their immense support and contribution in my research work.

Last but not the least, I would like to thank my family and friends for their care and encouragement during my research work.

# Dedication

## Dedicated to My Parents and My Family Members

# Table of Content

# List of Figure

# List of Table

# Abstract

*Garcinia cowa* has risen the interest of scientists and researchers since some of the active constituents and phytochemicals found on this plant are known to exert many beneficial effects. Garcinia is a rich source of secondary metabolites.

Phytochemical investigations of the plant parts indicated that leaves are the best source of secondary metabolites, providing glycosides, alkaloids and xanthones respectively. Many of the isolated compounds have a wide range of pharmacological activities including anticancer, anti-inflammatory, antibacterial, anti-fungal, antidepressant and antioxidant.

The aim of the present study was to evaluate the antioxidant and antimicrobial activity of petroleum ether ( pet ether ) extract of *Garcinia cowa*.

The antioxidant activity was measured by DPPH and total Phenol test. The $IC_{50}$ values of DPPH test was 242.188μg/ml for pet ether extract of *Garcinia cowa* leaves. The Total Phenol content was 107.47±30.62 mg/g equivalent to Gallic Acid for pet ether extract of *Garcinia cowa* leaves.

The antimicrobial activities of pet ether extract of *Garcinia cowa* leaves were tested against ten microorganisms by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method.In case of 300 μg/disc, the highest antimicrobial activity was shown against *Pseudomonas aureus*. The diameter of the zone of inhibition was 12mm compared to the 30 mm of diameter of the zone of inhibition of the standard zentamycin. It showed the moderate activity against *Bacillus subtilis, Salmonella paratyphi, Salmonella typhi, Shigella dysenteriae, Vibrio parahemolyticus*, *Staphylococcus aureus* and *Escherichia coli*. In case of 600 μg/disc, the highest antimicrobial activity was shown against *Bacillus megaterium* and *Sarcina lutea*. The diameter of the zone of inhibition was 12mm compared to the 30 mm of diameter of the zone of inhibition of the standard Gentamycin.

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

# Introduction

## 1.1 General Introduction

Plants and man are inseparable. Plants existed on the earth in the geological past from 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. WHO consultative body of medicinal plants has formulated a definition of medicinal plants in the following way "A medicine plant is any plant which in one or more of its organ, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs" (Sofowora, 1982).

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants use were instinctive, as is the case with animals. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illness or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plant' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16$^{th}$ century, plants had been the source of treatment and prophylaxis. Nonetheless, the decreasing efficacy of synthetic drugs and increasing contraindications of their usage make the usage of natural drugs topical again (Khan *et al.,* 2005).

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past of which there is sample evidence from various

sources like written documents, preserved monuments and even original plant medicines. Awareness of medicinal plan usage is a result of the many years of struggles against illness due to which man learned to pursue drugs in barks, seeds, fruit bodies and other parts of the plants. Contemporary science has acknowledged their active action and it has included in modern pharmacotherapy a range of drugs of plant origin known by ancient civilization and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life (Rates, 2001).

In Bangladesh, there are about 297 Unani, 204 Ayurvedic and 77 Homeopathic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi-processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations. The market value of drugs produced by these industries from medicinal plants is about TK. 300 cores (The Daily Jugantor, 2003).

### 1.1.1 History of medicine

From the ancient time treatment of diseases was associated with supernatural, superstitions along with the use of plant and other mineral core. The treatment pattern was changed with the development of knowledge and sciences. At the beginning history the treatment was confined within the treatment of wounds.

The Egyptians and Babylonians both introduced the concepts of diagnosis, prognosis, and medical examination. The first ever known medical ethics was developed by Greeks. The Hippocratic Oath, still taken by doctors up to today, was written in the 5th century BCE.

In the medieval era, surgical practices inherited from the ancient masters were improved and then systematized in Rogerius's The Practice of Surgery. Systematic training at the universities for the physicians began around the years 1220 in Italy. During the Renaissance, understanding of anatomy improved, and the microscope was invented.

The germ theory of disease in the 19th century led to cures for many infectious diseases. Public health measures were developed especially in the 19th century as the rapid growth of cities required systematic sanitary measures. Advanced research centers opened in the early 20th century, often connected with major hospitals.

The mid-20th century new biological treatments was discovered, such as antibiotics. These advancements, along with developments in chemistry, genetics, and lab technology (such as the x-ray) led to modern medicine. Medicine was heavily professionalized in the 20th century, and new careers opened to women as nurses from the 1870s and as physicians especially after 1970. The 21st century is characterized by very advanced research involving numerous fields of science (Jackson M. *et al.,* 2011).

## 1.1.2 Medicinal Plants from Ancient Times

Archaeological evidence indicates that the use of medicinal plants dates at least to the paleolithic, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years, to the Sumerians, who created lists of plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations or on rare occasions found in medical jars containing trace amounts of herbs. The earliest known Greek herbals were those of Diocles of Carystus, written during the 3$^{rd}$ century B.C and one by Krateuas from the 1$^{st}$ century B.C. only a few fragments of these works have survived intact but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Seeds likely used for herbalism have been found in the archaeological sites of Bronze Age China dating from the Shang Dynasty. Over a hundred of the 224 drugs mentioned in the Huangdi Neijing, an early Chinese medical text are herbs. Herbs were also common in the medicine of ancient India, where the principle treatment for diseases was diet. *De Materia Medica* by Pedanius Dioscorides, a Roman physician is a particularly important example of such writings. The documentation of herbs and their uses was a central part of both Western and Eastern medical scholarship through to the 1600s and these works played an important role in the development of the science of botany.

Human beings have used plants for the treatment of diverse aliments for thousands of years. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements, since they cannot afford the products of Western pharmaceutical industries, together with their side effects and lack of healthcare facilities. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health but they may not understand the science behind these medicines but knew that some medicinal plants are highly effective only when used at therapeutic doses.

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

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

## 1.1.3 Traditional Medicine

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Traditional medicine that has been adopted by other populations is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal

products that contain parts of plants or other plant materials as active ingredients. In Bangladesh, a long tradition of indigenous herbal medicinal systems, based on the rich local plant diversity, is considered as very important component of the primary health care system. About 75 per cent populations are getting their primary health care through traditional and herbal medicines. We can provide the better service through implementation of standardized. The large number of industries (traditional and herbal factories) have been established in this country for producing Ayurvedic, Unani, Homeopathies and Herbal medicines. It has been estimated that Bangladesh has a market of about 330-core taka worth traditional and herbal prod-ucts annually. Bangladesh has near about 550 medical plants. More than 300 of such medicinal plants are now in common use in the preparation of traditional medicines in Bangladesh. Traditional medicine systems of Unani, Ayurvedic, Homoeopathy and herbal among such people (Sitesh C Bachar, 2013).

1. One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:
    - ***Folk medicine,*** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like bloodletting, bones setting, hot and cold baths, therapeutic fasting and cauterization.
    - ***Religious medicine,*** which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods etc. and
    - ***Spiritual medicine,*** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.
2. The other is the improved and modified form based on the following two main traditional system:
    - ***Unani****- Tibb or Graeco-Arab system*, which has been developed by the Arab and Muslim scholars from the ancient Greek system and

- ***Ayurvedic system***, which is the old Indian system, based on the Vedas the oldest scriptures of the Hindu saints of the Aryan age.

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them (Ghani A, 1998).

## 1.1.4 Significance of Medicinal Plants to Mankind

If we only consider the impact of the discovery of the penicillin obtained from microorganism on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drug prescribed worldwide come from plants, 121 such active compounds being in current use. In order to obtain a cure for any illness, to experience new sensation or just because of simple curiosity, these type of plants have been very as necessary as appealing to us, since ancient times. For example Echinacea was used by North America native population to cure wounds or for sneak bites. It's important as a remedy to boost immunity is very present in recent days. Echinacea is considered a very efficient natural antibiotic for people using medicinal plants. The importance of medicinal plants is most evident in the present in developing countries. In Pakistan an estimated 80% of people depend on these for treatment, 40% in China. In technologically advanced countries like the United States an estimated 60% of the medicinal plants is commonly used to combat certain diseases. In Japan there is more demand for medicinal plants. Modern medicine, through the clinical analysis, has clarified the validity of those plants that had used the traditional method based on trial and error. Many proved to be valid, others proved to be harmless, others potentially dangerous. The biochemical analysis could determine which are the main components of medicinal plants. - So-called active ingredients. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. In addition, compounds such as muscarine, physostigmine cannabinoids, colchicine and esters, all obtained from plants are important tools used in pharmacological, physiological and biochemical studies (Williamson E *et al.,* 1996).

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

- A future medicine bank to discover-There are about half a million flowering plants, most of which has not been investigated and the principles of which could be crucial in the healing of current or future diseases.
- Synergic medicine- The components of the plants have a synergistic effect by interacting all at once, so their uses can complement or enhance or neutralize their potential negative effects.
- Support of Medical Officer- The treatment of complex diseases in some cases may require the support of the medicinal properties of plants or derivatives that provide them.
- Preventive Medicine- Finally, we must not forget the preventive nature that plants have with respect to the onset of disease. In this sense the plants to overcome the chemical remedies that are applied mainly when the disease has appeared. It has been established as the ingestion of natural foods can prevent many diseases i.e., reduce the side effect of synthetic treatment.

## Table 1.1 Name & Medicinal Uses of Some Common Plants in Bangladesh

(Dixie G. *et al,* 2003)

| Common name | Botanical Name | Parts Used | Medicinal Use |
|---|---|---|---|
| Amla | *Emblica officinalis* | Fruit | Vitamin - C, Cough , Diabetes, cold, Laxativ, hyper acidity |
| Ashok | *Saraca Asoca* | Bark Flower | Menstrual Pain, uterine, disorder, Diabetes |
| Aswagandha | *Withania Somnifera* | Root, Leaf's | Restorative Tonic, stress, nerves disorder, aphrodisiacs. |
| Bael | *Aegle marmelous* | Fruit, Bark | Diarrhea, Dysentry, Constipation. |

| | | | |
|---|---|---|---|
| Chiraita | *Swertia Chiraita* | Whole Plant | Skin Disease, Burning, sensation, fever. |
| Kalmegh | *Andrographis Paniculata* | Whole Plant | Fever, weakness, release of gas. |
| Tulsi | *Ocimum sanclum* | Leaves/Seed | Cough, Cold, bronchitis, expectorant |
| Neem | *Azardirchata - indica* | Rhizome | Sedative, analgesic, epilepsy, hypertensive. |
| Satavari | *Asparagus Racemosus* | Tuber, root | Enhance lactation, general weakness, fatigue, cough. |

## 1.1.5 Advantages of Drug Discovery from Natural Resources

Usage of botanical sources as starting point in the drug development program is associated with few specific advantages:

- Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active com-pounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. At certain time point afterward, one may attempt upon synthesis of active molecule and reduce pressure on the resource. Drug development from *Rauwolfia serpentina*, *Digitalis purpurea* etc. in the past fall under this category of approach.
- Sometimes, such approaches lead to development of novel podophyllin derived from *Podophyllum hexandrum* was faced with dose-limiting toxicities. Such limitations could be overcome to a great extent by semi-synthesis of etoposide, which continues to be used in cancer therapy today. Similar was the case with camptothecin (originally isolated from

Camptotheca sp. and subsequently from Mappia sp.), which led to development of novel anticancer molecules like topotecan and irinotecan.

- Natural resources as starting point has a bilateral promise of delivering the original isolate as a candidate or a semi-synthetic molecule development to overcome any inherent limitations of original molecule (Katiyar C. *et al.,* 2012).

## 1.1.6 Global Scenario of Medicinal Plants

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal medicine is a common element in Ayurvedic, homeopathic, naturopathic, Oriental, traditional, Native America &Indian medicine. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. The present total global herbal market1 is of size 62.0 billion dollars, in this India's contribution is only one billion dollars. European Union is the biggest market with the share 45% of total herbal market. North America accounts for 11%, Japan 16%, ASEAN countries 19% and rest of European Union 4.1% 158.Increasing demand for botanical remedies is both a national and international trend. In fact, the global herbal supplement and remedies market is expected to reach $93 billion by 2015, according to a new report by San Jose, CA-based Global Industry Analysts, Inc 159. In the U.S., sales of herbal supplements increased more than 3% in 2010, reaching more than $5 billion, according to a new report published in Herbal Gram, ABC's quarterly journal 159. As of January 2011, the U.K. Medicines and Healthcare products Regulatory Agency (MHRA) recorded 187 traditional herbal applications, of which 84 have been granted 159. While Europe may represent one of the largest regional markets, "in terms of growth rate, the Asia-Pacific market, led largely by China and India, is set to pave the way with the highest CAGR (compound annual growth rate) of [nearly 11%] through 2015," according Global Industry Analysts' most recent report 159. India's share in medicinal plant export in global trade is very low about 8.13% as against 28% of China 160. The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually worldwide. Global market size for herbal and medicinal plants is estimated at US$ 60 billion and is projected to reach US$ 5 trillion by 2050 (source WHO 2003). About 75% to 80% of the total exports of crude drugs come from India 160.The trend growth

rate of India from the year 1991 to 2002 shows 4.95% growth of world export value of medicinal plants. Similarly the trend growth rate of China from 1991-2002 is 7 (Rates, 2001).

## 1.1.7 Medicinal Plant in Bangladesh

In an estimate, the international market of medicinal plants related to trade stood at 60 billion US Dollar per year. The demand for medicinal plants based raw materials are growing at an approximate rate of 10-15% per year internationally. Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jumping in volume of plants materials trade within and across the country. Bangladesh there is no systematic cultivation process or conservation strategies about medicinal plants. The local people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation. This knowledge now under threat to extinction. This is a very alarming situation with regard to natural growth of medicinal plants in the wilderness in this country. In this scenario, the survey on "Traditional and industrial use and market Scenario of Medicinal plants in Bangladesh." has been conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh. We have found that there is worth of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products. This research aimed at documenting the 'Present Status and Market Scenario of Medicinal Plants' in Bangladesh. Our research finding shows that 84.1% of the respondent use medicinal plants in health care. 18.3% of the villagers use Kabirazi in the disease in medium category.55.0% of our respondent's source of knowledge of using medicinal plant is family where 34.7% gained knowledge from neighbor. Only 14.3% of the respondents are involved with trading of medicinal plant. About 10.4% of the villagers are involved in cultivation, collection or business of medicinal plant. From the survey report it has been found that 46.6% industries are using above 60% of imported medicinal plants as their raw materials and 53.3% of the industries are using below 40%. The study revealed that 86.7% industries are importing Indian raw materials, 53.3% are importing the Pakistani one and very few of them are importing the raw materials from Nepal, Iran and Korea. According to the response of shop owners, the local raw materials of their products are mostly coming from 5 different areas of the country. Among those 90% are coming from Chittagong and again 76.6%

from Tangail, 30% from Gazipur and another 30% from Khulna. In this scenario, appropriate steps must therefore be taken immediately in order to save this situation with regard to growth, conservation and supply of medicinal plants in the country. The best possible way of doing this is to bringing this more and more of these plants under planned cultivation. The cultivation of medicinal plants in Bangladesh will lead to the conservation and also protect the biodiversity. Ecological and biotic factors are suitable in Bangladesh for the cultivation of medicinal plants. We have been successful to sensitize the policy makers. In Bangladesh there is no facilities and skilled manpower for the processing of MPs. Our research is now aiming to develop processing unit and to train the garden owner for skilled manpower to value addition of MP, which will create the income generating women in rural areas. It is estimated that some 12,500 tons of dried medicinal plant material produced in Bangladesh is sold. These products are worth some Taka 255 million ($4.5 million) to the rural economy and around Tk.330M ($5.8M) at the factory rate/wholesale. The 5,000 tons of imported medicinal plants cost around Taka 480 million ($8 million). It is believed that there are around 350 inter-district beparis who are serviced by 6,000 to 10,000 local collectors, pikers and growers. In total there are said to be around 200 Unani and 200 Ayurvedic registered factories, plus some 70 homeopathic factories. Collectively they will employ 2,000 to 4,000 people. In addition, there are said to be 5,000 qualified and 80,000 unqualified herbal practitioners in the country ( Bregum F, 2010).

## 1.2 Approaches for Isolation of Active Compound from Medicinal plant

### 1.2.1 Random Approach

Two approaches have been followed for screening of the plants selected randomly for the purpose of new drug discovery.

- Screening for selected class of compounds like alkaloids, flavonoids, etc.- While this route is simple to perform, however, it is flawed in the sense that it provides no idea of the biological efficacy. However, chances of getting novel structures cannot be denied following this approach.

- Screening of randomly selected plants for selected bioassays- Central Drug Research Institute, a premier R and D organization of Council of Scientific and Industrial Research of India, followed this approach about three decades ago. They screened almost 2000 plants for biological efficacy. However, the screening did not yield any new drug. National Cancer Institute (NCI) of National Institute of Health, USA, studied about 35,000 plant species for anticancer activity, spending over two decades from 1960 to 1980. It resulted in proving two success stories, which were those of paclitaxel and camptothecin. This route, therefore, has been applied for both focused screening as well as general screening, showing some success in focused screening. If target-based bioassays are used, e.g. screening against PTP1B, chances of success would probably be more. This approach, however, needs a huge library of extracts, which very few organizations in the world are having (Katiyar C. *et al.,* 2012).

## 1.2.2 Ethnopharmacology Approach

The approach of ethnopharmacology essentially depends on empirical experiences related to the use of botanical drugs for the discovery of biologically active NCEs. This process involves the observation, description, and experimental investigation of indigenous drugs, and is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines like anthropology, archaeology, history, and linguistics. This approach based on ethnomedicinal usage history has seen some success, e.g. *Andrographis paniculata* was used for dysentery in ethnomedicine and the compounds responsible for the activity were isolated as andrographolide. Morphine from *Papaver somniferum*, Berberine from *Berberis arisata* and Picroside from *Picrorrhiza kurroa* are some example of this approach. Some of the plants which are not selected on the basis of ethnomedical use also had some success stories like L-Dopa from *Mucuna prurita* and Paclitaxel from *Taxus brevifolia* (Katiyar C. *et al.,* 2012).

## 1.2.3 Traditional System of Medicine Approach

Countries like India and China have a rich heritage of well-documented traditional system of medicine in vogue. Though these codified systems of medicine use largely botanical sources as medicines, however, these stand apart from ethnomedicine specifically on three accounts:

- The ethnomedicinal practice is based on empirical experiences. On the other hand, these codified systems built up the empirical practices on strong conceptual foundations of human physiology as well as of pharmacology (though the tools of their investigations in those times were far different from the existing ones).

- The pharmaceutical processes have been more advanced as against the use of crudely extracted juices and decoctions in ethnomedicinal practices. Due to this phenomenon, the concept of standardization was known to the system.

- They are well documented and widely institutionalized. On the other hand, the ethnomedicinal practices are localized and may be largely controlled by few families in each of the community.

However, in terms of historicity, ethnomedicinal practices might be older than codified systems of medicine (Katiyar C. *et al.,* 2012).

## 1.3 Procedure for Development

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

1. Selection and correct identification of the proper medicinal plant.
2. Extraction with suitable solvent(s).
3. Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.
4. Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.

5. Repeated fractionation of active fractions to isolate pure compound(s).
6. Elucidation of chemical structure of pure compound(s) using spectroscopic methods.
7. Evaluation of biological activity of pure compound(s).
8. Toxicological tests with pure compound(s).
9. Production of drug in appropriate dosage forms.

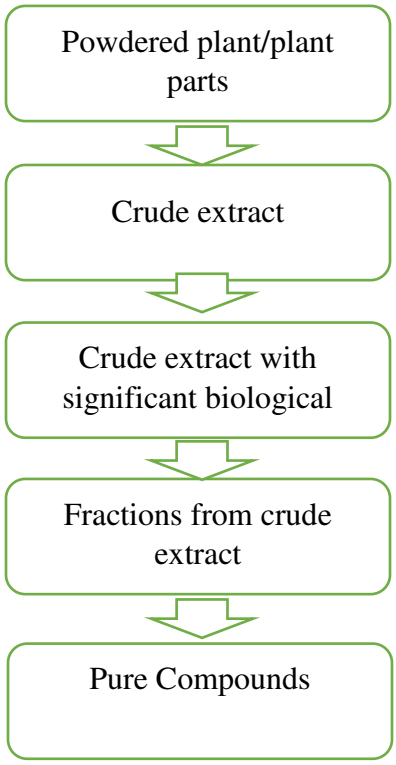



**Figure 1.1 : Schematic Diagram of Development of Procedure**

### 1.3.1 Selection of Plant Species

- Preliminary screening of traditionally used plants
- Review literature and scientific result
- Authentication of data for their validity and comprehensiveness

### 1.3.2 Evaluation of Toxicity

- Gather data concerning toxicity and if demonstrate no toxicity then proceed to next step

- If toxicity data is not exit, select an appropriate test for toxicity analysis
- Develop and prepare bioassay protocol for safety and toxicity

### 1.3.3 Preparation of Plant Sample and Element Analysis

- Collection of plant sample
- Extraction
- Analysis for elemental contents

### 1.3.4 Biological Testing

- Selection of appropriate biological test
- Development protocol for biological test
- Analyze biological activity in- vivo
- Determine type and level of biological activity

### 1.3.5 Isolating Active Compounds

- Isolating and characterization of compounds responsible for observed biological activity.
- Evaluation of active compounds singularly and in combination with others to explore existence of activity and/or synergy of biological effect

### 1.3.6 In-Vivo Analysis

- Use animal model for bioactivity analysis of active compounds
- Analyze again safety and toxicity but in in-vivo
- Conduct human studies

### 1.3.7 Commercialization

- Develop appropriate dose delivery system
- Analyze cost-effectiveness, Sustainable industrial production (Katiyar C. *et al.,* 2012).

## 1.4 Extraction of Crude from Medicinal Plants

Extraction involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. So the products obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. There are several extraction procedures for obtaining active component from medicinal plants.

### 1.4.1 Extraction Methods

#### 1.4.1.1 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

#### 1.4.1.2 Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs. Digestion This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

#### 1.4.1.3 Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents. This process is typically used in preparation of Ayurvedic extracts called “quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

### 1.4.1.4 Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product.

### 1.4.1.5 Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

### 1.4.1.6 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (co-solvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt.

### 1.4.1.7 Phytonics Process

A new solvent based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant materials offer significant environmental advantages and health and safety benefits over traditional processes for the production of high quality natural fragrant oils, flavors and biological extracts. Advanced Phytonics Limited

(Manchester, UK) has developed this patented technology termed "phytonics process". The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment (Handa S. *et al.* 2008).

## 1.4.2 Parameters for Selecting An Appropriate Extraction Method

- If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. For example, lupeol is the active constituent of *Crataeva nurvala* and, for its extraction, hexane is generally used. Likewise, for plants like *Bacopam onnieri* and *Centella asiatica*, the active constituents are glycosides and hence a polar solvent like aqueous methanol may be used.
- If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. For thermostable constituents, Soxhlet extraction (if non-aqueous solvents are used) and decoction (if water is the menstruum) are useful.
- Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g. flavonoids and phenyl propanoids.
- In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.
- Standardization of time of extraction is important, as: Insufficient time means incomplete extraction. If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.
- The number of extractions required for complete extraction is as important as the duration of each extraction.
- The quality of water or menstrum used should be specified and controlled.
- Concentration and drying procedures should ensure the safety and stability of the active constituents. Drying under reduced pressure is widely used. Lyophilization, although expensive, is increasingly employed.
- The design and material of fabrication of the extractor are also to be taken into consideration.

- Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documented to monitor the quality of different batches of the extracts (Sasidharan S. *et al.*, 2010).

## 1.5 Phytochemical Screening

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. Terpenoids exhibit various important pharmacological activities i.e.,anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anesthetic agents and are found in medicinal plants. The leaves of the selected medicinal plants were washed, air dried and then powdered. The aqueous extract of leaf samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, phlobatannins, reducing sugar, flavonoids and alkaloids were found to be present in afore mentioned medicinal plants. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants of Mardan will be very useful in the curing of various diseases of this region (Wadood A. *et al.,* 2013).

## 1.6 Antioxidant Potential of Medicinal Plants

Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. Consumption of antioxidant-

enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals. Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C. The antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life-threatening diseases, including neurodegenerative and cardiovascular disease (Kasote M. *et al.,* 2015).

## 1.6.1 DPPH Radical Scavenging Activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to yellow colored product, diphenyl picryl hydrazine, by the addition of the extract in a concentration dependent manner. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Our results revealed that the methanolic extract of TPL had a similar free radical scavenging activity when compared with standard BHT. Polyphenol contents and tocopherols scavenge the DPPH radicals by their hydrogen donating ability. The results obtained in this study suggest that all the extracts from *T. pallida* showed radical scavenging activity by their electron transfer or hydrogen donating ability. Total polyphenols content and radical scavenging antioxidant activity are highly correlated (Rahman M. *et al.,* 2015).

## 1.6.2 Classification of Antioxidant

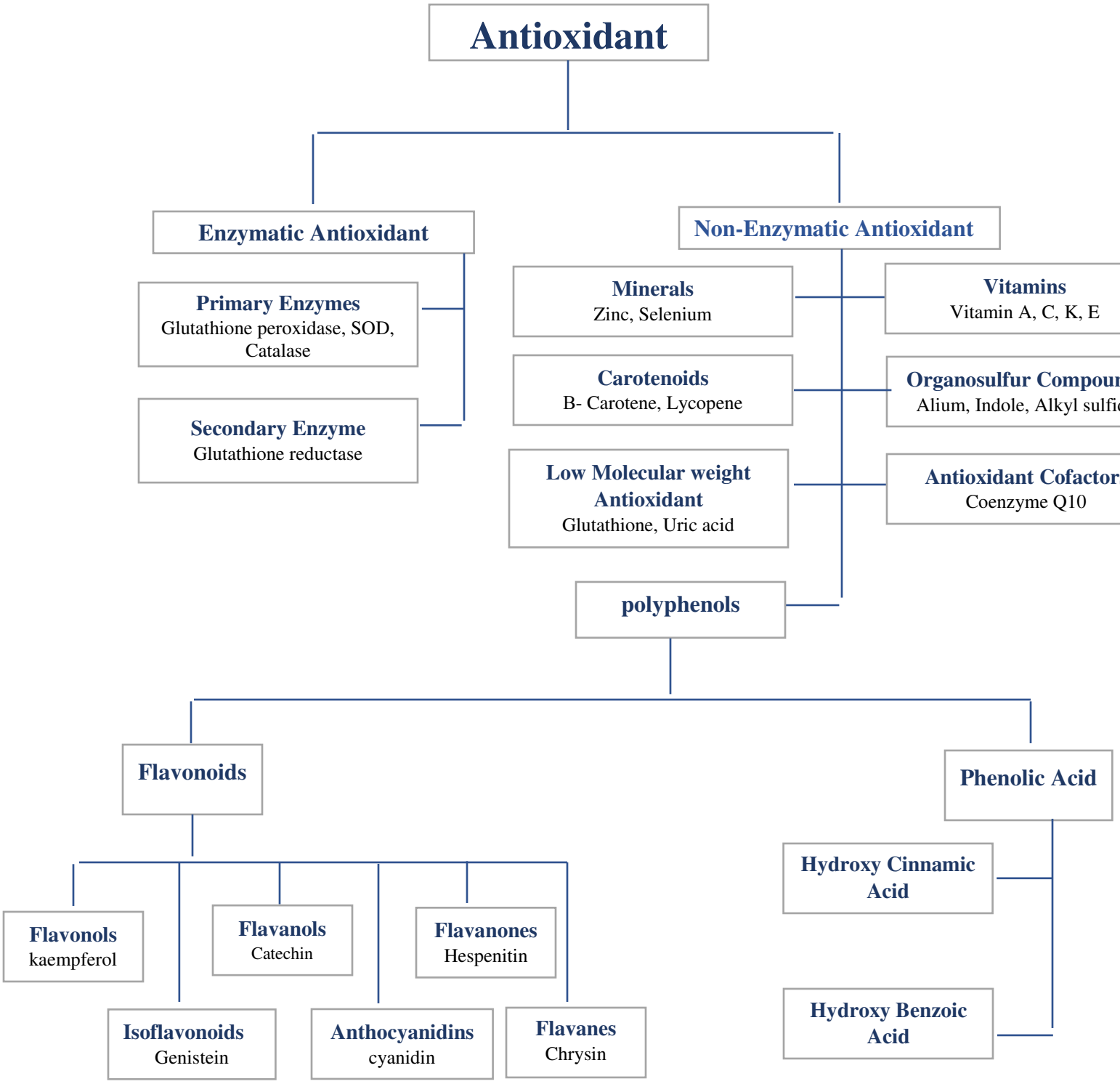



**Figure 1.2 : Schematic Diagram of Classification of Antioxidant**

## 1.6.3 Free Radicals and Oxidative Stress

Free radicals produced in our body due to aerobic respiration and substrate oxidation, can cause oxidative stress which may contribute to the development of several diseases including cancer, Alzheimer's disease, aging, diabetes, Parkinson disease and atherosclerosis. Overproduction of free radicals in our bodies may be increasing due to pollution and other external factors, and their removal by our antioxidant systems may be lower than before due to a number of factors related to our lifestyle among others. Oxidative stress causes serious damage to important cellular macromolecules such as protein and DNA. However, the production of free radicals can be balanced by antioxidant actions of endogenous enzymes as well as natural and synthetic antioxidants. Antioxidants exert its action through several mechanisms including prevention of chain initiation, chelating of transition metal ion catalysts, decomposition of peroxidases, prevention of continued hydrogen abstraction and radical scavenging (Rahman M. *et al.,* 2015).

## 1.6.4 Disease-fighting antioxidants

A diet high in antioxidants may reduce the risk of many diseases, including heart disease and certain cancers. Antioxidants scavenge free radicals from the body cells, and prevent or reduce the damage caused by oxidation. The protective effect of antioxidants continues to be studied around the world. For instance, men who eat plenty of the antioxidant lycopene (found in tomatoes) may be less likely than other men to develop prostate cancer. Lutein, found in spinach and corn, has been linked to a lower incidence of eye lens degeneration and associated blindness in the elderly. Flavonoids, such as the tea catechins found in green tea, are believed to contribute to the low rates of heart disease in Japan (Rahman M. *et al.,* 2015).

# 1.7 Antimicrobial Screening

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used testing methods include broth microdilution or rapid automated instrument methods that use commercially marketed materials and devices. Manual

methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods.

## 1.7.1 Emergence of Antimicrobial Resistance and the Rationale for Performing Susceptibility Testing

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed e.g., continued penicillin susceptibility of *Streptococcus pyogenes* . Susceptibility testing of individual isolates is important with species that may possess acquired resistance mechanisms (Reller L. *et al.,* 2009).

## 1.7.2 Commonly Used Susceptibility Testing Methods

### 1.7.2.1 Broth Dilution Tests

One of the earliest antimicrobial susceptibility testing methods was the macro broth or tube-dilution method. This procedure involved preparing two-fold dilutions of antibiotics (eg, 1, 2, 4, 8, and 16 μg/mL) in a liquid growth medium dispensed in test tubes. The antibiotic containing tubes were inoculated with a standardized bacterial suspension of $1–5\times10^5$CFU/mL. Following overnight incubation at 35°C, the tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the minimal inhibitory concentration (MIC). The precision of this method was considered to be plus or minus 1 two-fold concentration, due in large part to the practice of manually preparing serial dilutions of the antibiotics. The advantage of this technique was the generation of a quantitative result (Reller L. *et al.,* 2009).

### 1.7.2.2 The Advantages of The Microdilution

This procedure includes the generation of MICs, the reproducibility and convenience of having pre-prepared panels, and the economy of reagents and space that occurs due to the

miniaturization of the test. There is also assistance in generating computerized reports if an automated panel reader is used (Reller L. *et al.,* 2009).

### 1.7.2.3 Antimicrobial Gradient Method

The antimicrobial gradient diffusion method uses the principle of establishment of an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility. The Etest (bioMérieux AB BIODISK) is a commercial version available in the United States. It employs thin plastic test strips that are impregnated on the underside with a dried antibiotic concentration gradient and are marked on the upper surface with a concentration scale. As many as 5 or 6 strips may be placed in a radial fashion on the surface of an appropriate 150-mm agar plate that has been inoculated with a standardized organism suspension like that used for a disk diffusion test. After overnight incubation, the tests are read by viewing the strips from the top of the plate. The MIC is determined by the intersection of the lower part of the ellipse shaped growth inhibition area with the test strip (Reller L. *et al.,* 2009).

### 1.7.2.4 Disk Diffusion Test

The disk diffusion susceptibility method is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1–2\times10^{8}$CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface. Plates are incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute or those included in the US Food and Drug Administration (FDA)-approved product inserts for the disks. The results of the disk diffusion test are “qualitative,” in that a category of susceptibility is derived from the test rather than an MIC. The advantages of the disk method are the test simplicity that does not require any special equipment, the provision of categorical results easily interpreted by all clinicians, and flexibility in selection of disks for testing (Reller L. *et al.,* 2009).

## 1.7.3 Interpretation of Susceptibility Test Results

The results of a susceptibility test must be interpreted by the laboratory prior to communicating a report to a patient's physician. Optimal interpretation of MICs requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites. This is best accomplished by referring to an expert source such as the CLSI, which publishes interpretive criteria for MICs of all relevant antibiotics for most bacterial genera. Indeed, both MIC values and disk diffusion zone diameters must be interpreted using a table of values that relate to proven clinical efficacy of each antibiotic and for various bacterial species. The CLSI zone size and MIC interpretive criteria are established by analysis of 3 kinds of data-

1. Microbiologic data, including a comparison of MICs and zone sizes on a large number of bacterial strains, including those with known mechanisms of resistance that have been defined either phenotypically or genotypically.
2. Pharmacokinetic and pharmacodynamic data.
3. Clinical studies results (including comparisons of MIC and zone diameter with microbiological eradication and clinical efficacy) obtained during studies prior to FDA approval and marketing of an antibiotic (Reller L. *et al.,* 2009).

## 1.7.4 Current Test Methods And Future Direction

The antimicrobial susceptibility testing methods described in this article provide reliable results when used according to the procedures defined by the CLSI or by the manufacturers of the commercial products. However, there is considerable opportunity for improvement in the area of rapid and accurate recognition of bacterial resistance to antibiotics. There is a need for development of new automated instruments that could provide faster results and also save money by virtue of lower reagent costs and reduced labor requirements. To accomplish this, it will likely be necessary to explore different methodologic approaches for detection of bacterial growth. The direct detection of resistance genes by polymerase chain reaction or similar techniques has limited utility, because only a few resistance genes are firmly associated with phenotypic resistance. There are hundreds of β-lactamases, and numerous mutations,

acquisitions, and expression mechanisms that result in fluoroquinolone, aminoglycoside, and macrolide resistance too many to be easily detected by current molecular techniques. Thus, it seems likely that phenotypic measures of the level of susceptibility of bacterial isolates to antimicrobial agents will continue to be clinically relevant for years to come (Reller *et al.,* 2009).

## 1.8 Review on Mangosteen (*Garcinia cowa*)

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

The fruit, capped by the prominent calyx at the stem end and with 4 to 8 triangular, flat remnants of the stigma in a rosette at the apex, is round, dark-purple to red-purple and smooth externally; 1 1/3 to 3 in (3.4-7.5 cm) in diameter. The rind is 1/4 to 3/8 in (6-10 mm) thick, red in cross-section, purplish-white on the inside. It contains bitter yellow latex and a purple, staining juice. There are 4 to 8 triangular segments of snow-white, juicy, soft flesh (actually the arils of the seeds). The fruit may be seedless or have 1 to 5 fully developed seeds, ovoidoblong, somewhat flattened, 1 in (2.5 cm) long and 5/8 in (1.6 cm) wide, that cling to the flesh. The flesh is slightly acid and mild to distinctly acid in flavor and is acclaimed as exquisitely luscious and delicious (Borsani C *et al.,* 2004).

### 1.8.1 Vernacular Name of *Garcinia cowa*

Bengali/vernacular name: Kau, Cowa, Kaglichu; Kao-gola (Chittagong)
Tribal name: Kao-gula (Chakma, Tanchangya), Tah Gala (Marma)
English name: Cow Tree

## 1.8.2 Taxonomical Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Clusiaceae

Genus: Garcinia

Species: *Garcinia cowa*

## 1.8.3 Growth Habit

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, 34 solitary or by 3-5 at the end of the branchlets. Berry the size of a lime, slightly 6-8 lobed, dull 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 A, 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).

## 1.8.4 Salient Feature of Family Clusiaceae

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

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

Scotch attorney, or cupey (Clusia rose), which is native to the Caribbean area, grows to about 10 meters (30 feet). It has leaves 10 cm (4 inches) long, flatly open flowers with six waxy, rosy-white petals, and many-seeded, multicelled, golfball-sized fruits. Like other species in the family, the fruits open and the valves spread widely like a star, exposing the succulent bright-orange tissue (arils) surrounding the seeds. Scotch attorney is planted as a beach shrub in areas exposed to salt spray. *C. grandiflora*, which is native to Suriname, has larger flowers and ivory-white central stamen masses. Many members of the genus Clusia begin as epiphytes, or air plants, and eventually send roots over the host tree to the ground. All of the 300 to 400 members of the genus are tropical American.Mammee Apple or mamey (Mammea americana), native to tropical America, produces a grapefruit-sized, rough, russet-skinned, edible fruit. The other members of the genus Mammea are tropical but especially common in Madagascar.

**Figure 1.3 : Whole Plant of *Garcinia cowa***

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

## 1.8.5 Morphology

The plants are small to medium sized evergreen trees which may grow up to 30 m in height and are widely distributed in the tropical and temperate regions of the world.

### 1.8.5.1 Fruits

The fruits and young leaves are edible with a sour taste. The bark is dark brown with a yellow latex. The fruits are globose (2.5-6.0 cm in size), green when young and dull orange or yellow atmaturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3- angled seeds (Sharmin, T. *et al.,* 2004).

**Figure 1.4 : Fruits of *Garcinia cowa***

### 1.8.5.2 Flowers

The plant has unisex flowers: yellow orange female flowers found at the end of branches and male flowers found along the branches as clusters. Flowers polygamous, 4-merous. Flowers borne on the axils of the fallen leaves. Male flowers in dense terminal or axillary clusters, hermaphrod its flowers.

**Figure 1.5 : Flowers of *Garcinia cowa***

### 1.8.5.3 Seeds

Seeds 4-8 per fruits, enclosed in a fleshy or succulent aril.

**Figure 1.6 : Seeds of *Garcinia cowa***

### 1.8.5.4 Leaves

Leaves (711x4-7) cm. broadly elliptical lanceolate, acuminate in apex. Midvein prominent, lateral vein not prominent. Leaf petiole size Short (0.30.6x0.6-0.8) cm. in size. The leaves are glossy, deep green, oblong and up to 6-15 cm in length and 2.5- 6.0 cm in width.


**Figure 1.7 : Leaves of *Garcinia cowa***

## 1.8.6 Chemical Constituent & Biological Activity

Many pharmaceutical drug discoveries originated from traditional folk medicine and its associated plant materials and bioactive secondary metabolites. The Genus Garcinia, belonging to the Family Clusiaceae which comprises about 300 species, have been widely investigated in terms of their bioactive ingredients. Native to Asia, Africa, South America and Polynesia. Twenty-nine species have been observed in Thailand, with 20, 13, 12, 7, 6 and 3 species found in the south, middle, north, east, north-east and west of the country respectively. Garcinia is a rich source of secondary metabolites, especially triterpenes, flavonoids, xanthones and phloroglucinols. The latter two groups are well recognized as cheomotaxonomic markers for this genus . Many of the isolated compounds have a wide range of pharmacological activities including anticancer, anti-inflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant and antioxidant. Many parts of *Garcinia cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an anti fever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant. The chemical composition and biological activities of various parts of *Garcinia 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.

The biological activities of the extracts from various parts of *Garcinia 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 *Garcinia cowa* were tested against four Gram-positive bacteria (*Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis* and *Staphylococcus aureus*) and one Gram-negative bacterium (*Escherichia coli*). Both extracts significantly inhibited bacterial growth of the Gram-positive bacteria (IC50s 15-30 □g/mL) but not *Escherichia coli* (IC50s 250-500 □g/mL). The extracts were also found to inhibit the growth of *Aspergillus flavus* ATCC 46283, a common fungal food contaminant which produces aflatoxin B1. The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (ca 40-60% at the same concentration). The methanol extracts of the leaves and twigs of *Garcinia cowa* were evaluated for their ability to inhibit low-density lipoprotein peroxidation

induced by copper ions. The twig extract had an IC50 value of 20.5 □g/mL and was more potent (higher % inhibition at 1000 □g/mL) than the leaf extract (IC50 not measured). The twig extract was more potent than the leaf extract on platelet aggregation of human whole blood induced by arachidonic acid, adenosine diphosphate and collagen. These activities may be due to the total phenolic content of these extracts, which were 19 and 61 mg of gallic acid equivalent per g of extract for the leaf and twig extracts respectively (Schimmel K. *et al,* 2004).

## 1.8.7 Classes of Compounds Isolated from Garcinia cowa

### 1.8.7.1 Depsidones

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, cowa depsidone was the first and only known depsidone from *Garcinia cowa*. It was isolated from the twig extract and showed cytotoxicity against NCI-H187 and MFC-7 cancer cell lines.

### 1.8.7.2 Flavonoids

Twelve flavonoids were isolated from *Garcinia cowa* with garccowasides A, B and C being first reported as new compounds. Of these compounds, only morelloflavone showed strong antioxidant activities. Phloroglucinols are based on a phloroglucinol or 1,3,5-benzenetriol core skeleton or its 1,3,5- cyclohexanetrione (phloroglucin) tautomer. The phloroglucinols found in *Garcinia cowa* have a benzoyl group and geranyl and polyprenyl units as substituent groups. So far, fifteen phloroglucinols have been obtained from the twig including six new compounds: guttiferone K, chamuangone, garcicowins A, B, C and D and nine known phloroglucinols: cambogin, guttiferones K, B and F, oblongifolins B, C, A and D and 30-epicambogin. Some of them showed selective cytotoxicity against two cancer cell lines (HT-29 and HCT-116) and normal colon cells (CCD-18Co). Guttiferone K and 30-epicambogin exhibited highest cytotoxicity against cancer cell line HT-29.

### 1.8.7.3 Xanthones:

Xanthones, with two aromatic rings linked via carbonyl and ether linkages, are the major components of the Garcinia genus. They are commonly found in several parts of *G. cowa*,

especially in the stem, fruit and latex. Thirty six xanthones (46% of the total isolated compounds) have been isolated and nineteen of them were first isolated from *Garcinia cowa*. They are cowagarcinone, cowaxanthone, cowanol, cowanin, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3- methyl-2-butenyl) xanthone, norcowanin, cowagarcinones A, B, E and D from the latex cowaxanthones B, C, D and E from the fruit 7- O-methylgarcinone E, 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3methylbutyl)xanthone, 4-(1,1-dimethyl-prop-2-enyl)-1,5,6-trihydroxy-3-methoxy-2-(3methylbut-2-enyl)xanthen-9(9H)-one and 1,5-dihydroxy-3methoxy-6′,6′-dimethyl-2Hpyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone from the stem and cowaxanthone F from the twig. Most of these xanthones showed interesting biological activities.

### 1.8.7.3.1 Antibacterial activity

Extracts of *Garcinia mangostana* showed inhibitory effects against the growth of *Staphylococcus aureus* NIHJ 209p and some of the components had activity against methicillin-resistant *Staphylococcus aureus* (MRSA). One active isolate, α-mangostin, a xanthone derivative, had a minimum inhibitory concentration (MIC) of 1.57-12.5 ug/ml. Other related xanthones were also examined to determine their anti-MRSA activity. The strong in-vitro antibacterial activity of xanthone derivatives against both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* suggested the compounds might find wide pharmaceutical use (Iinuma *et al.,* 2009).

### 1.8.7.3.2 Anti-inflammatory activity

Eight xanthones: cowaxanthones A, B, C and D, α-mangostin, mangostanin, cowanol and cowanin were tested for their anti-inflammatory activity using the ethyl phenylpropiolate induced ear edema assay. All xanthones except cowanol were more active than the standard drug, phenylbutazone. Antimalarial activity Five xanthones isolated from the stem bark: 7-O-methylgarcinone, α-mangostin, cowaxanthone, cowanol and cowanin had significant in vitro antimalarial activity against Plasmodium falciparum with IC50 values ranging between 1.5-3.0 µg/mL. Anticancer activity Six xanthones: cowaxanthone, cowanol, cowanin, norcowanin, 3,6-di-Omethyl-γmangostin and dulxanthone isolated from twig were evaluated for their cytotoxicity against NCI-H187, KB, MFC-7 and/or HepG2 cell lines. Cowaxanthone, cowanin, norcowanin and 3,6-di-O-methyl-γ-mangostin exhibited significant cytotoxicity against the NCI-H187 cell line with 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).

### 1.8.7.3.3 Antifungal activity

The antifungal activity of several xanthones isolated from fruit hulls of *Garcinia mangostana* (collected from Tamil Nadu, India) and some derivatives of mangostin against *Fusarium oxysporum* f.sp. vasinfectum, Alternaria tenuis [*A. alternata*] and Drechslera oryzae [*Cochliobolus miyabeanus*] was evaluated. The natural xanthones inhibited the growth of all the fungi. Substitution in the A and C rings modified the bioactivities of the compounds (Geetha *et al,* 2009).

### 1.8.7.3.4 Cosmetic uses

The technical data and scientific studies confirm that this extract is an excellent choice for antibacterial, antifungal and anti-inflammatory effects on the skin. These are exactly the conditions encountered in acne-prone skin where soaps, creams and washes ideally suit the use of the extract. Made into an ointment, it is applied on eczema and other skin disorders [Morton]. The traditional oral use also suggests the use of this plant in herbal toothpastes for good oral hygiene.

## 1.8.8 Medicinal Uses

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

- A root decoction is taken to regulate menstruation. A bark extract called "amibiasine", has been marketed for the treatment of amoebic dysentery.
- The rind of partially ripe fruits yields a polyhydroxy-xanthone derivative termed mangostin, also ß-mangostin. That of fully ripe fruits contains the xanthones, gartanin, 8-disoxygartanin, and normangostin. A derivative of mangostin, mangosine, 6-di-O-glucoside, is a central nervous system depressant and causes a rise in blood pressure.

**Table 1.2 Morphology Character & Ethanobotany of Genus *Garcinia*** (Baruah S. *et al.,* 2012)

| Particulars | *Garcinia pedunculata* | *Garcinia paniculata* | *Garcinia Morella* Desr. | *Garcinia lanceaefolia* | *Garcinia xanthochymus* Hook. |
|---|---|---|---|---|---|
| Local name | Borthekera(Ass), Prumang (K) | Schopatenga(Ass), Marlo (K) | Kuji-thekera (Ass) | Rupohi-thekera (Ass) | Tepor-tenga (Ass) |
| Distribution | North East India. | Assam, Meghalaya and Nagaland | Assam, Arunachal Pradesh, Meghalaya and Nagaland | Assam, Meghalaya and Nagaland. | Lower altitude of N.E. India. |
| Habit | An evergreen tree, rather short spreading branches | A small evergreen tree | A small middle sized evergreen tree. | A small evergreen tree. | An evergreen middle size tree. |
| Leaves | Leaves (1330x 1521) cm., obovate or oblanceolate, rigid subcoriaceous | Leaves (1115x4.5-6.5) cm. elliptic or oblanceolate acuminate, subcoriaceous | Leaves (1011x5-7) cm. elliptic to ovatelanceolate, obtusely acuminate | Leaves (4.56x2.5-3.5) cm. lanceolate long acuminate. | Leaves (2532x4-8) cm. Narrowly oblong or oblong lanceoate |
| Midvein | Midvein stout prominent, lateral vein distinct | Midvein stout prominent, lateral vein distinct | Midvein prominent, lateral vein slightly prominent | Midvein prominent, lateral vein not prominent. | Midvein prominent, lateral vein distinct. |

| | | | | | |
|---|---|---|---|---|---|
| Leaf petiole size | Petiole long (1.5-2.5x1.21.3) cm. in size | Petiole long (2.22.8x1.1-1.4) cm. in size | Short (0.41x0.4-0.8) cm. in size. | Short (0.51x0.3-0.5) cm. in size. | Long (1.5-1.8x11.5) cm. in size. |
| Pedicels size | Pedicels long and thickened (3-5x4-4.5) cm. in size | Pedicels short (0.40.6x1-1.2) cm. in size | Short pedicels | Pedicels short (0.4-0.5x0.50.8) cm. in size. | Pedicels short and thickened. |
| Flowers | Flowers polygamous, 4 merous; male flowers large pale green, stamens many. | Flowers polygamous , 4-merous, male flower white sepals and petals 4 each, stamens numerous. | Flowers polygamous tetramerous; male flowers generally together, in axils of fallen leaves, hermaphrodite flowers solitary. | Flowers polygamous, 4 merous. Male flowers 1-2 terminal; sepals thick, petals smaller, stamens many. Hermaphrodite flowers terminal of axillary. | Flowers polygamous pentamerous ; male flowers borne on the axils of the fallen leaves. Sepals -5, petals-5, stamens -5. Stigma oblique. |
| Fruits | Fruits large, yellow in color when ripe. Mature fruits (78.2x25-29.8) cm. in size. Fresh wt. of the mature fruits av. 500 gm. | Fruits small (3-41x1215) cm in size, cherry, yellow, succulent with granular stigma. | Fruits 1.5-2 cm. in diameter globose or slightly elongated, yellow when in ripe. | Fruits small ovovoid about 2 cm. in diameter, orange-yellow in color. | Fruits 3.5-6 cm. in diameter, subglobose, pointed, golden yellow in color when ripe. |
| Seeds | Seeds 4-8 per fruits, enclosed in a fleshy or succulent aril. | Seeds generally 4, enclosed in a pulpy aril | Seeds 4, testa dark brown. | Seeds 6-8. | Seeds 2-6. |
| Flowering time | Throughout the year. | December-February. | February-March. | February-March. | March-May |
| Fruiting time | January-April. | March-April. | April-June | June- July. | October-February. |

| | | | | | |
|---|---|---|---|---|---|
| Ethanobotany | The fruits are acidic and edible, preserved after sundried for local consumption. The old dried fruits are good for dysentery, digestive and cooling. The fruits are also used as fixative or as a mordant for saffron dye. Wood is hard has potential value used for making house, wooden furniture and traditional rice mill "Dheki" preparation | The ripe fruits are eaten and very delicious. Leaves are used to treated roundworm. Wood is moderately hard used for house building, firewood (Dutta 1985). | The fruits are eaten raw or dried, good for dysentery. A commercial source of 'gamboge' occurs as a yellowish color; oil and juice of fruits are cooling for fever, diabetes and jaundice. | The fruits are acidic and pentamerous eaten raw or dried, good for dysentery; the gum resin is called 'gamboge' is used as medicine and as yellow dye; oil and juice of fruits are cooling for fever, jaundice and urinary troubles | The fruits are acidic and edible. The ripe fruits used for making jams, delicious chutney in Assamese household. A sherbet made from ripens and dried fruits are given in dysentery. Bark of the tree and latex of unripe fruits are used to make yellow dye. Woods is hard, good for making house. |

# Literature Review on *Garcinia cowa*

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

## 2.1 The Use of *Garcinia* Extract (Hydroxycitric Acid) As a Weight loss Supplement: A Systematic Review and Meta-Analysis of Randomised Clinical Trials

The aim of this systematic review is to examine the efficacy of *Garcinia* extract, hydroxycitric acid (HCA) as a weight reduction agent, using data from randomized clinical trials (RCTs). Electronic and nonelectronic searches were conducted to identify relevant articles, with no restrictions in language or time. Two independent reviewers extracted the data and assessed the methodological quality of included studies. Twenty-three eligible trials were identified and twelve were included. Nine trials provided data suitable for statistical pooling. The meta-analysis revealed a small, statistically significant difference in weight loss favoring HCA over placebo (MD: -0.88 kg; 95% CI: -1.75, -0.00). Gastrointestinal adverse events were twice as common in the HCA group compared with placebo in one included study. It is concluded that the RCTs suggest that *Garcinia* extracts/HCA can cause short-term weight loss. The magnitude of the effect is small, and the clinical relevance is uncertain. Future trials should be more rigorous and better reported (Igho, O. *et al.,* 2010).

## 2.2 Microencapsulation of *Garcinia Cowa* Fruit Extract and Effect of Its Use on Pasta Process and Quality

Microencapsulation is employed to protect bioactive ingredients in foods and is also used for their controlled release at targeted sites. Hydroxycitric acid ((-)-HCA) is present in the fruits of certain species of *Garcinia* and it has been studied extensively for its unique regulatory effect on fatty acid synthesis, lipogenesis, appetite, and weight loss. Since hydroxycitric acid is hygroscopic in nature, it is very difficult to convert liquid extract from the fruits of *Garcinia* into dried powder. Hence, microencapsulation of *Garcinia cowa* fruit extract was performed in a pilot-scale co-current spray dryer with whey protein isolate as a wall material. In this study, two different wall-to-core ratios (1:1 and 1.5:1) and dryer outlet temperatures (90 and 105°C) were

used for assessing the encapsulation efficiency. The results in this study showed that the microencapsulation efficiency (based on HPLC analysis) and antioxidant properties (based on 2,2-diphenyl-1-picrylhydrazyl assay) were higher at 90°C outlet temperature of the spray dryer using 1.5:1 wall-to-core ratio feed. Further, the spray-dried powders were incorporated into pasta processing and evaluated its quality characteristics. The results of this study demonstrated that incorporation of powder spray-dried at 90°C outlet temperature with 1.5:1 wall-to-core pasta exhibited higher antioxidant activity as well as better cooking and sensory characteristics (Pillai D. *et al.,* 2012).

## 2.3 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 °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 °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 *Garcinia cowa* (Jena B. *et al.,* 2002).

## 2.4 Cytotoxic Compounds from the Leaves of *Garcinia cowa* Roxb

Compounds from the leaves of methanol extract of *Garcinia cowa* was isolated and their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines was evaluated. The dichloromethane fraction was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature these compounds were elucidated as methyl 2,4,6- trihydroxy-3-(3-methylbut-2-enyl) benzoate (1), garcinisidone-A (2) and methyl 4,6dihydroxy-2-(4-methoxy-5- (3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4-dienyloxy)-3-(3-methylbut-2-enyl) benzoate (3). Compound 1, 2 and 3 had IC50 value of 21.0 ± 10.2 µM, 21.2 ±8.4 µM and 17.2 ± 6.2µM against MCF-7, while only

compound (2) was found to be in active against H-460 with IC50 value of 18.1 ± 6.7 μM. Conclusion: The results indicate that G. cowa leaves could be important sources of natural cytotoxic compounds and only compound (2) had activity against H-460 cell lines (Wahyuni F. *et al.,* 2015).

## 2.5 Antioxidant and Antiplatelet Aggregation Properties of The Bark Extracts of *Garcinia pedunculata* and *Garcinia cowa*

The bark extract of *Garcinia pedunculata* and *Garcinia cowa* which is abundant in the Northeastern regions of India, were screened for their antioxidant and in vitro antiplatelet aggregating activities. By β-carotene linoleate model for antioxidant assay, acetone extract of *Garcinia pedunculata* and hexane extracts of *Garcinia 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 *Garcinia pedunculata* and hexane extract of *Garcinia cowa* exhibited higher antiplatelet aggregation capacity towards ADP induced platelet aggregation (IC50 0.16 and 0.43 ug, respectively) than other extracts (Sharma A. *et al.,* 2014).

## 2.6 Cowaxanthone F and Other Anti-inflammatory and Antioxidant Compounds from *Garcinia cowa*

A new tetra oxygenated 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 (diphenyl picrylhydrazyl), hydroxyl, and superoxide radicals; only morelloflavone (2) and morelloflavone-7 "-O-glucoside(4) exhibited high potency. Eight tetra oxygenated xanthones from the fruits of *Garcinia cowa*, cowaxanthones A-D (6-9), cowanin (15), alphamangostin (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 α-mangostin (16) exhibited significant anti-inflammatory activity when compared to phenylbutazone, while cowaxanthone A (6), mangostanin (17), and cowanol (18) showed less activity (Panthong K. *et al.,* 2009).

## 2.7 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 *Garcinia cowa* and *Garcinia pedunculata*, respectively. However, the hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* exhibited marked inhibitory effect against all the test organisms and were more effective than that of *Garcinia 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 *Garciia cowa* and *Garcinia pedunculata* (Negi P, *et al.,* 2008).

## 2.8 Antibacterial Tetra Oxygenated Xanthones from The Immature Fruits of *Garcinia cowa*

A phytochemical investigation of the acetone extract from the immature fruits of *Garcinia cowa* led to the isolation of two novel tetra oxygenated xanthones, garcicowanones A (1) and B (2), together with eight known tetra oxygeanted xanthones. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their antibacterial activity against *Bacillus cereus* TISTR 688, *Bacillus subtilis* TISTR 008, *Micrococcus luteus* TISTR 884, *Staphylococcus aureus* TISTR 1466, *Escherichia coli* TISTR 780, *Pseudomonas aeruginosa* TISTR 781, *Salmonella typhimurium* TISTR 292 and *Staphylococcus epidermidis* ATCC 12228. α-Mangostin showed potent activity (MIC 0.25–1 µg/mL) against three Gram–positive strains and garcicowanone A and β-mangostin exhibited strong antibacterial activity against *Bacillus cereus* with the same MIC values of 0.25 µg/mL (Auranwiwat C. *et al.,* 2014 ).

## 2.9 Kaennacowanols A–C, Three New Xanthones And Their Cytotoxicity from The Roots of *Garcinia cowa*

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

## 2.10 Sub-acute Toxicity Study of The Ethyl Acetate Fraction of Asam Kandis Rinds (*Garcinia cowa* Roxb.) on The Liver and Renal Function in Mice

The present study investigated the sub acute toxicity of the ethyl acetate fraction of asam kandis (*Garcinia cowa Roxb*) Rinds in mice. Sub acute toxicity study was carried out by giving orally at dose 500, 1000 dan 2000 mg / kg BW extract to five mice at 21 days. Animals were observed individually for any clinical signs of toxicity or mortality for 14 days. Measured parameters were SGPT levels, serum creatinine levels, weight ratio of liver and kidney. Extract was given orally at dose 500, 1000 and 2000 mg/kg BW for 21 days. Observations were done on day 8th, 15th and 22th using blood serum, liver and kidneys of mice. Data were analyzed by using two-way ANOVA followed by Duncan's Multiple Range Test. The ethyl acetate fraction of *G. cowa* at doses 500, 1000 and 2000 mg/kg BW gave significant effect on increasing SGPT levels and decreasing levels of serum creatinine ($p < 0.05$). The length of treatment gave significant effect on decreasing levels of serum creatinine, weight ratio of liver and kidney ($p < 0.05$). The dosage of the ethyl acetate fraction of asam kandis rinds provides significant effect on the SGPT and serum creatinine levels of male white mice. The duration of administration of ethyl acetate fraction of asam kandis rinds provides significant effect on serum creatinine levels, the weight ratio of liver and kidney organ of male white mice (Wahyuni F. *et al.,* 2017).

## 2.11 Seed Dispersal, Seed Predation And Seedling Spatial Pattern of *Garcinia cowa* (Guttiferae)

Seed dispersal is a multi-step process, which may include fruit removal, seed dissemination, post-dispersal seed predation, potential secondary dispersal, seed germination, and seedling establishment. Previous studies often focused on only one or a few of these stages. In this study, we demonstrate the complexity of the seed dispersal process of *Garcinia cowa*, a common climax under canopy tree in seasonal rain forests of SW China (Liu Y. *et al.,* 2002).

## 2.12 Two New Xanthones from The Stems of *Garcinia cowa*

Two new xanthones, 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone and 1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl)xanthone, have been isolated together with six known xanthones: 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone, dulxanthone A, 1,5,6-trihydroxy-3,7-dimethoxyxanthone, 1,7-dihydroxyxanthone, 1,3,5-trihydroxy-6-methoxyxanthone, 1,3,6,7-tetrahydroxyxanthone, from the stems of *Garcinia cowa* (Guttiferae) (Shen J. *et al.,* 2006).

## 2.13 Bioactive Prenylated Xanthones from The Young Fruits and Flowers of *Garcinia cowa*

Five new xanthones, Garciniacowaones A-E (1-5) together with 14 known xanthones, 6-19 were isolated from the young fruits and fresh flowers of *Garcinia cowa*. The structure of 1-5 were elucidated by analysis of their 1D and 2D NMR spetra and mass spectrometric data. The compounds 1-19 were tested in vitro for their antimicrobial activity and for their ability to inhibit α-glucosidase. Compounds 16 and 17 showed the most potent α-glucosidase inhibitory activity with $IC_{50}$ values of 78 ±0.5 and 8.7 ± 0.3 μM respectively. Compounds 8,9 and 19 showed antibacterial activity against *Bacillus subtilis* TISTR 088 with identical MIC values of 2 μg/mL, while 8, 10 and 19 exhibited antibacterial activity against *Bacillus cereus* TISTR 688 with identical MIC values of 4 μg/mL (Sriyatep T. *et al.,* 2015).

## 2.14 Cytotoxic Properties and Complete Nuclear Magnetic Resonance Assignment of Isolated Xanthones from The Root of *Garcinia cowa* Roxb.

The ground air-dried root was sequentially macerated with hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and methanol. The DCM soluble extract was fractionated by vacuum liquid chromatography, column chromatography, and radial chromatography over silica gel with hexane, EtOAc and methanol as eluent in progressively increasing polarity manner; to yield three compounds. Their structures were elucidated based on their spectroscopic data and their comparison with those of the literature. The cytotoxicity of isolated compounds was carried out against human cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. The extract was added at various concentrations (0.1, 1, 10 and 100 mg/ml). The level of cytotoxicity was determined by calculating the level of $IC_{50}$ that was based on the percentage of the cell death following the 24 h incubation with the extract. Phytochemical study on the roots of *Garcinia cowa* yielded rubraxanthone (3), cowanine (4) and 1,5-dihydroxyxanthone (5). Compound 4 with an $IC_{50}$ value of 4.1 ± 1.0 μM, 5.4 ± 2.3 μM and 11.3 ± 10.0 μM against MCF-7, H-460, and DU-145, respectively while compound 3 was found to be in active. The results indicate that *Garcinia cowa* roots could be important sources of natural cytotoxic compound (Wahyuni F. *et al,* 2016).

## 2.15 Antiaflatoxigenic and Antioxidant Activities of *Garcinia* Extracts

The effect of hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* on the growth and aflatoxin production in *Aspergillus flavus* was studied using peanut powder as a model food system. The growth of *Aspergillus flavus* was completely inhibited by the hexane and chloroform extracts from *Garcinia cowa* and chloroform extract from *Garcinia pedunculata* at 3000 ppm concentration, which was considered as the minimum inhibitory concentration (MIC). The MIC for the hexane extract of *Garcinia pedunculata* was at 4000 ppm. Both the extracts from *Garcinia cowa* inhibited aflatoxin $B_1$ production up to 100% at a lower concentration of 2000 ppm. It was observed that, at lower concentration of the extracts from *Garcinia cowa* and *Garcinia pedunculata*, the degree of inhibition of aflatoxin production was much higher than the inhibition of fungal growth. The hexane and chloroform extracts from *Garcinia cowa* and *Garcinia pedunculata* were also studied for their antioxidant capacity by the formation of phosphomolybdenum complex at 100 ppm concentration and reducing power

by potassium ferricyanide reduction method at various concentrations. Hexane and chloroform extracts from *Garcinia cowa* showed higher antioxidant capacity than *Garcinia pedunculata* extracts. Similarly, both the extracts from *Garcinia cowa* showed higher reducing power than the extracts from *Garcinia pedunculata*. The antiaflatoxigenic activities of the extracts from *Garcinia cowa* and *Garcinia pedunculata* may be due to their effective antioxidative properties, which could suppress the biosynthesis of aflatoxin (Joseph G S. *et al.,* 2005).

# Methodology

## 3.1 Collection & Preparation of Plant Material

*Garcinia cowa* plant was collected in the month of April, 2017 from Comilla, Bangladesh 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 24 hours 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.

## 3.2 Washing and Drying of *Garcinia cowa* Plant

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc. within them. 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 50°C for 2 hours.

## 3.3 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 500 gm which was measured using electronic balance and it was found to be 500 gm.

## 3.4 Extraction of the Dried Powdered Sample

The fine powder of *Garcinia cowa* leaves was dissolved in 2.5 L pet ether and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered

particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

## 3.5 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.

## 3.6 Evaporation and Condensation of the leaf extracts

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

## 3.7 Principle of a Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure"). Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts. A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Büchi in 1957. Other common evaporator brands are Heidolph, LabTech, Stuart, Hydrion Scientific, SENCO, IKA and EYELA. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L,50L) versions are used in pilot plants in commercial chemical operations.

**Figure 3.1 : Rotary Evaporator**

## 3.8 Theory of Phytochemical Screening

### 3.8.1 Materials (Reagents and Tools) Used

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

| | |
|---|---|
| Hager's reagent (Saturated solution of picric acid). | Burner |
| Dragendroff's reagent (Bismuth sub nitrate and acetic acid solution)- All for alkaloid tests. | |

### 3.8.2 Test Compounds

Petroleum Ether ( Pet ether ) extract of leaves of *Garcinia cowa.*

### 3.8.3 Preparation of Sample Solution

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

### 3.8.4 Phytochemical Tests

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

- **Molisch's test for carbohydrates:** Two drops of Molisch's reagents were added to about 5 mg of the extract in 5 ml aqueous solution in a test tube. 1 ml of conc. $H_2SO_4$ was allowed to flow down the side of the inclined test tube so that the acid formed a layer beneath the aqueous solution without mixing with in. a red ring was formed at the common surface of the two liquids which indicated the presence of carbohydrate. On standing or shaking a dark-purple solution was formed. Then the mixture was shaken and diluted with 5 ml of water. Dull violet precipitate was formed immediately.
- **General test for glycosides:** A small amount of extract was dissolved in 1ml of water then few drops of aqueous NaOH solution was added. A yellow color was developed in the presence of glycosides.
- **Test for glycosides*:*** A small amount of extract was dissolved in water and alcohol then boiled with Fehling's solution. Any brick-red precipitation was noted. Another portion of extract was dissolved in water and alcohol and boiled with a few drops of dilute $H_2SO_4$. The acid was neutralized with NaOH solution and boiled with Fehling's solution. A

brick-red precipitation was produced in this experiment which showed the presence of glycosides in the extract.

- **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.
- **Tests for alkaloid*:*** A small volume of each extract was neutralized by adding 1 or 2 drops of dilute $H_2SO_4$. This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation was observed:
  a) **Mayer's reagent**: Formation of white and cream color precipitate indicated the presence of alkaloids.
  b) **Hager's reagent**: Formation of yellow crystalline precipitate indicated the presence of alkaloids.
  c) **Wagner's reagent**: Formation of brownish-black ppt indicated the presence of alkaloids.
  d) **Dragendroff's reagent*:*** Formation of orange or orange-red precipitate indicated the presence of alkaloids.

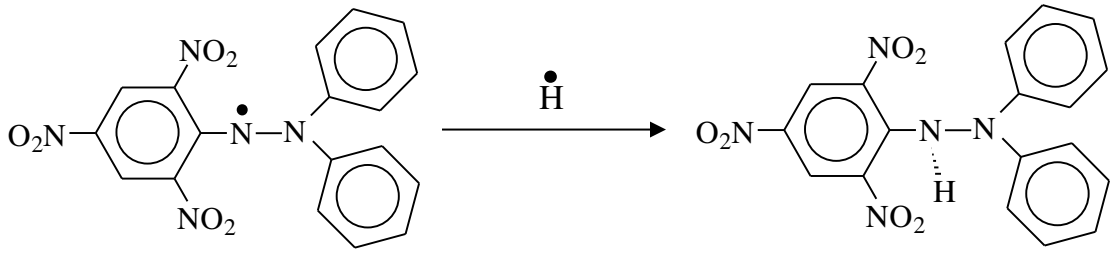
- **Test for saponins*:*** about 0.5 ml of extract was shaken vigorously with water in a test tube. If a forthing was produced and it was stable for 1-2 minutes and persisted on warming, it was taken as preliminary evidence for the presence of saponins.
- **Test for flavanoids*:*** A few drops of conc. HCl was added to a small amount of an extract. Immediate development of a red color indicated the presence of flavonoid.
- **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.
- **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.

# 3.9 Determination of Antioxidant property

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

### 3.9.1.1 Principle

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




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

| 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 |

### 3.9.1.2 DPPH Solution

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

### 3.9.1.3 Preparation of Standard/ Extract Solution

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

## Table 3.1 Preparation of Standard / Extract Solution For DPPH

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

### 3.9.1.4 Procedure

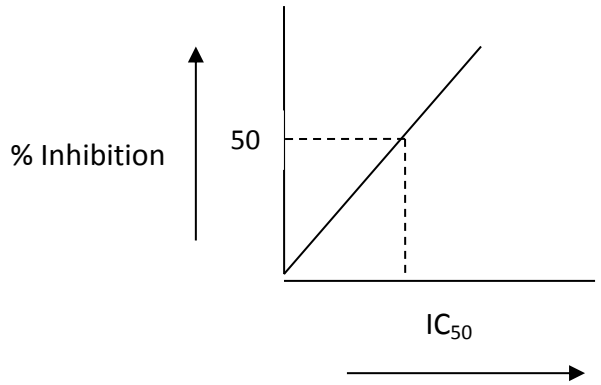
- The stock solution is serially diluted to achieve the concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml
- Each test tube contains 1ml of each concentration and is properly marked
- 2 ml of 0.004% DPPH solution in the solvent is added to each test tube to make the final volume 3 ml (caution: DPPH is light sensitive, so making the solution and adding it to the test tubes should be done in minimum light exposure)

- Incubate the mixture in room temperature for 30 minutes in a dark place
- Then the absorbance is measured at 517 nm against dilute extract solution in the solvent

#### 3.9.1.5 Calculation

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

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




## 3.9.2 Determination of Total Phenolics Content (Velioglu *et al.,* 1998).

#### 3.9.2.1 Principle

The content of total phenolic compounds of plant extracts was determined as described previously (Velioglu*et al.,* 1998) using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton*et al.,* 1999). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Vinson *et al.,* 2005).

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

easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI): Mo(VI) + $e^-$ → Mo (V)

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

### 3.9.2.2 Preparation of 7.5% Sodium Carbonate Solution

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

### 3.9.2.3 Preparation of Standard Solution

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

## Table 3.2 Preparation of Standard Solution For Phenolic Content

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

| | | | | |
|---|---|---|---|---|
| 12.5 | - | 1 ml (25 µl/ml) | 1000 | 2 |
| 6.25 | - | 1 ml (12.5 µl/ml) | | 2 |

### 3.9.2.4 Preparation of Extract Solution

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

## Table 3.3 Preparation of Extract Solution

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

### 3.9.2.5 Experimental Procedure

1. 1.0 ml of plant extract (200µg/ml) or standard of different concentration solution was taken in a test tube.
2. 5 ml of Folin-Ciocalteu (Diluted 10 fold) reagent solution was added to the test tube.
3. 7.5% Sodium carbonate solution (4 ml) was added to the same test tube and mixed well.
4. Test tubes containing standard solutions were incubated for 30 minutes at 20°C to complete the reaction but the test tubes containing extract solution were incubated for 1 hour at 20°C to complete the reaction.
5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
6. A typical blank solution contained the solvent used to dissolve the plant extract.

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

$$C = (c \times V)/m$$

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

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

V = the volume of extract in ml

m = the weight of crude plant extract in gm

## 3.10 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).

### 3.10.1 Materials

#### 3.10.1.1 Microorganisms

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

#### 3.10.1.2 Test Organisms

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

### 3.10.1.3 List of Test Bacteria

| Gram positive Bacteria | Gram negative Bacteria |
|---|---|
| *Bacillus cereus*<br>*Bacillus subtilis*<br>*Staphylococcus aureus*<br>*Proteus mirrabillis* | *Escherichia coli*<br>*Salmonella typhi*<br>*Pseudomonas aeruginosa*<br>*Serratiamarcescens* |

### 3.10.1.4 Culture Media And Chemicals

- Nutrient agar media
- Ethanol
- Chloroform

### 3.10.1.5 Equipments

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

### 3.10.1.6 Test Materials

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

## 3.10.2 Methods

### 3.10.2.1 Culture Preparation

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

## Table 3.4 Composition of Nutrient Agar Media (1000 ml)

| Ingredients | Amount |
|---|---|
| Beef extract | 3.0 gm |
| Peptone | 5.0 gm |
| Agar | 15.0 gm |
| Sodium chloride | 0.5 gm |
| 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 $25^0$C) 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

### 3.10.2.2 Sterilization Procedure

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

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

**Figure 3.2 : Laminar Air Flow**


**Figure 3.3 : Auto Clave**

### 3.10.2.3 Preparation of Subculture

In an aseptic condition under laminar air hood cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop tohave fresh pure cultures. The inoculated strains were then incubated for 24 h at $37^0$C for their optimum growth. These fresh cultures were used for the sensitivity test.

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

#### 3.10.2.5 Preparation of Discs

a) 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.

b) 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.

#### 3.10.2.6 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 petri dish 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.

### 3.10.3 Placement of Disc and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 40˚C for about 24 h. Finally the plates were kept in an incubator at 30˚C for 24 hr.

**Figure 3.4 : Incubator**

### 3.10.4 Determination of Zone of Inhibition

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

**Figure 3.5 : Zone Of Inhibition**

# Results & Discussion

## 4.1 Phytochemical Screening of Pet Ether Extract of *Garcinia cowa* Leaves

### Table 4.1 Result of Phytochemical Screenig

| Chemical constituents | Result |
|---|---|
| Carbohydrate | - |
| Glycoside | + |
| Alkaloid | + |
| Flavonoid | - |
| Saponin | - |
| Steroid | - |
| Tannin | + |

## 4.2 Antioxidant Property of Pet Ether Extract of *Garcinia cowa* Leaves

### 4.2.1 DPPH Test of *Garcinia cowa*

### Table 4.2 Result of Absorbance And % of Inhibition of *Garcinia cowa* Leaves And Ascorbic Acid

| Serial no | Concentration | Absorbance of Sample | Absorbance of Ascorbic Acid | % of Inhibition sample | % of Inhibition Ascorbic Acid |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 6.25 | 0.238 | 0.314 | 45.63758 | 29.75391 |
| 3 | 12.5 | 0.228 | 0.224 | 47.65101 | 49.88814 |
| 4 | 25 | 0.226 | 0.098 | 48.99329 | 78.07606 |
| 5 | 50 | 0.215 | 0.078 | 49.217 | 82.55034 |

| | | | | | |
|---|---|---|---|---|---|
| 6 | 100 | 0.196 | 0.019 | 49.66443 | 95.74944 |
| 7 | 200 | 0.14 | 0.014 | 49.88814 | 96.86801 |
| 8 | 400 | 0.09 | 0.011 | 53.02013 | 97.53915 |

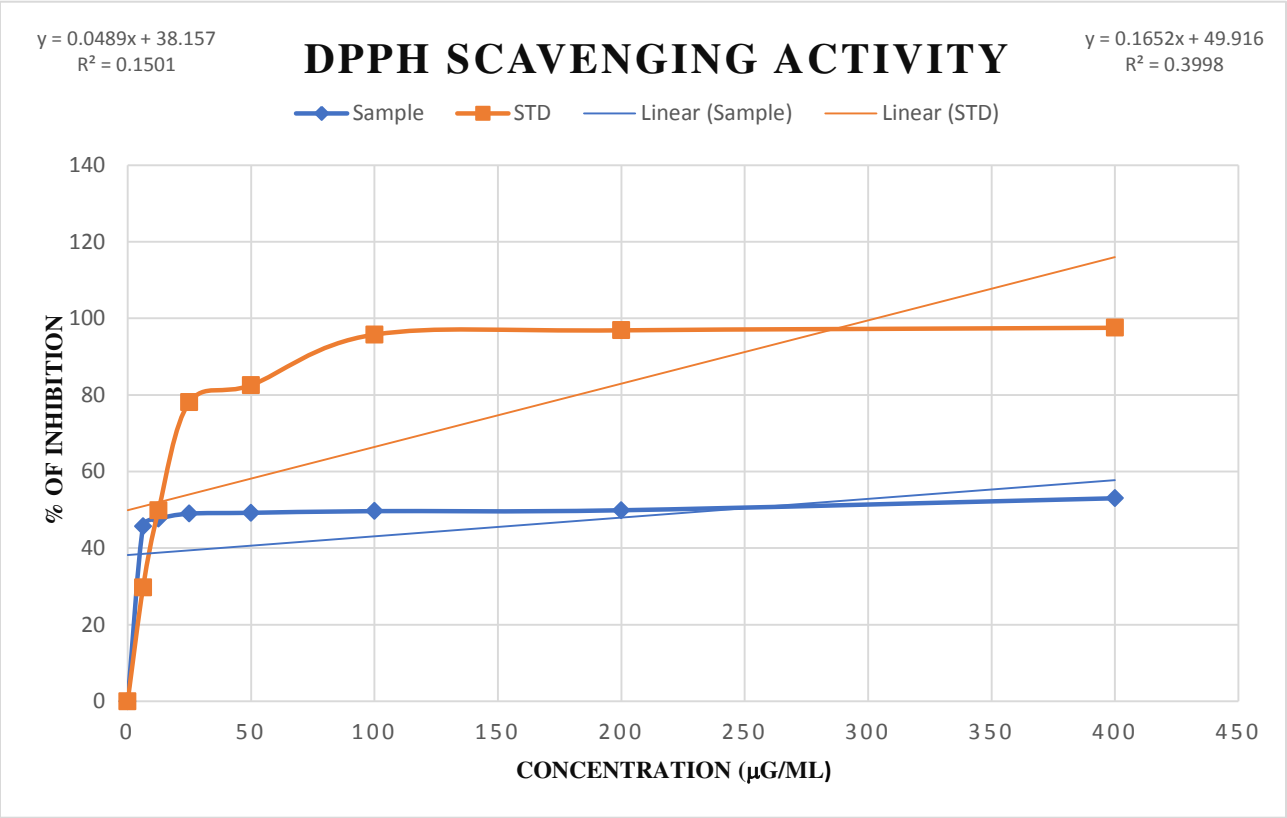



**Figure 4.1 : DPPH Scavenging Activity of Pet Ether Extract of *Garcinia cowa* Leaves And Ascorbic Acid**

**Table 4.3 Result of DPPH Test of Pet Ether Extract of *Garcinia cowa* Leaves And Ascorbic Acid**

| Name | Regression line | $IC_{50}$ value (µg/mL) | $R^2$ line |
|---|---|---|---|
| Pet ether extract of *Garcinia cowa* leaves | Y=0.0489x+38.157 | 242.188 | 0.1501 |
| Ascorbic acid | Y=0.1652x+49.916 | 0.508 | 0.3998 |

### 4.2.2 Total Phenolic Content of *Garcinia cowa*

**Table 4.4 Result of Absorbance of Pet Ether Extract of *Garcinia cowa* Leaves**

| Test tube no | Absorbance of sample |
|---|---|
| 1 | 0.214 |
| 2 | 0.311 |
| 3 | 0.301 |

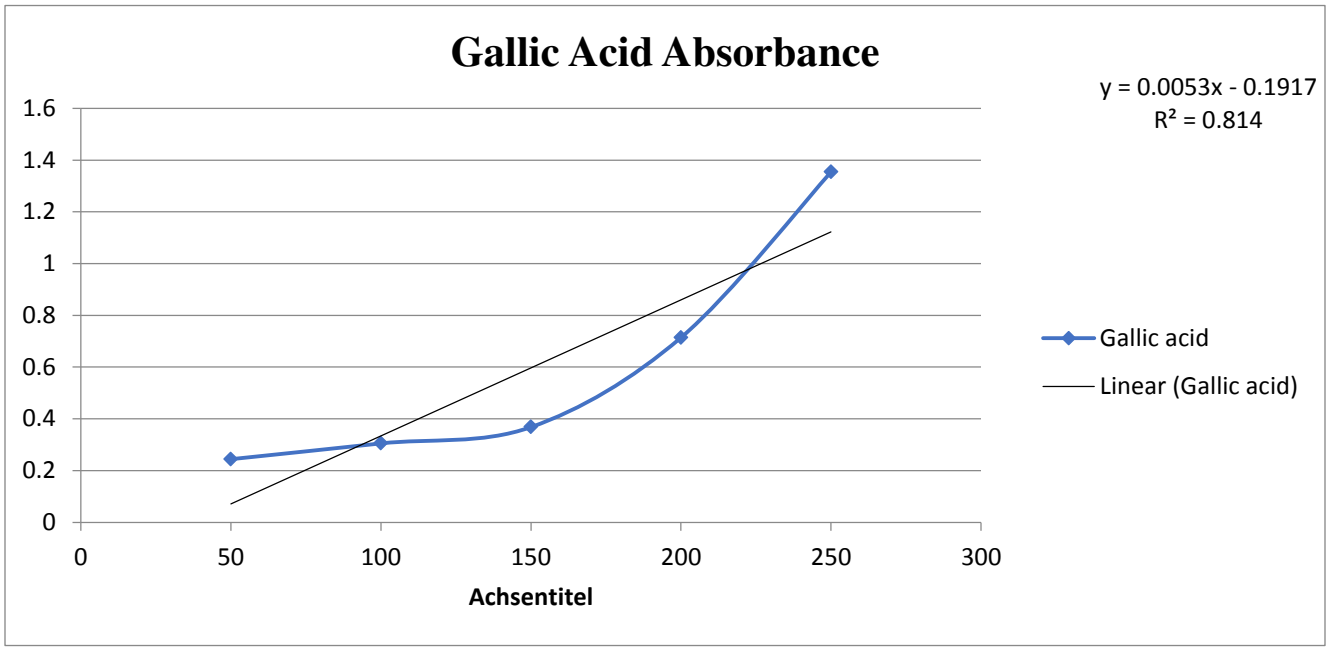



**Figure 4.2 : Standard Curve of Gallic Acid**

**Table 4.5 Result of Total Phenolic Content of Pet Ether Extract Of *Garcinia cowa* Leaves**

| Serial no | Absorbance of pet ether extract of *Garcinia cowa* leaves | Standard equation | Value of X | Mean | Standara deviation | Total phenolic content (mg/gm) |
|---|---|---|---|---|---|---|
| 1 | 0.214 | | 81 | | | |
| 2 | 0.311 | y=0.0053x-0.1917 | 100.4 | 93.27 | 10.67 | 93.27±10.67 |
| 3 | 0.301 | | 98.4 | | | |

## 4.3 Antimicrobial Screening of Pet Ether Extract of *Garcinia cowa* Leaves

**Table 4.6 Result of Zone of Inhibition of Pet Ether Extract of Garcinia cowa Leaves And Gentamycin**

| Name of Bacteria | Zone of Inhibition of sample (300 µg/disc) (mm) | Zone of Inhibition of Sample (600 µg/disc) (mm) | Zone of Inhibition of Gentamycin (30 µg/disc) (mm) |
|---|---|---|---|
| *Bacillus megaterium* | 10 | 12 | 30 |
| *Bacillus subtilis* | 8 | 9 | 30 |
| *Salmonella paratyphi* | 7 | 7 | 30 |
| *Salmonella typhi* | 6 | 8 | 30 |
| *Vibrio parahemolyticus* | 6 | 7 | 30 |
| *Staphylococcus aureus* | 6 | 7 | 35 |
| *Escherichia coli* | 6 | 7 | 30 |
| *Shigella dysenteriae* | 5 | 11 | 35 |
| *Pseudomonas aureus* | 6 | 12 | 30 |
| *Sarcina lutea* | 10 | 12 | 30 |

## 4.4 Discussion

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

In the previous study of literature review, 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 °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 °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 *Garcinia cowa.* (Jena, *et al,;* 2002).

In this study pet ether extract of the leaves of *Garcinia cowa* showed positive result in the tests of Glycoside, Alkaloid and Tannin compounds.

The reducing power of pet ether extract of leaves of *Garcinia cowa* was determined by comparing it with the standard ascorbic acid using the 1,.1-diphenyl-2-picrylhydrazyl (DPPH) reduction method. The present result suggest that the tested plant extract has potent antioxidant property. It has % inhibition of 84% compared to 92% of this by standard. Since a variety of constituents is present in the extract studied. Reducing power of extract was very potent and the power of extract was increased with quantity of sample. The plant extract could reduce the most DPPH, which had a lesser reductive activity than the standard of Ascorbic acid. It becomes difficult to describe all properties selectively to any one group of constituents without further studies , which are beyond the scope of this paper. So , further extensive investigations are necessary to find out the active principles present in this plants.

The content of total phenolic compounds of plant extracts was determined as described previously (Velioglu*et al.,* 1998) using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton*et al.,* 1999). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Vinson *et al.,* 2005).

In this study, $IC_{50}$ values of DPPH tests were 242.188 µg/ml for pet ether extract of *Garcinia cowa* leaves. The Total Phenol contents 107.47±30.62 mg/g equivalent to Gallic Acid for pet ether extract of *Garcinia cowa* leaves.

The antimicrobial activity of the pet ether extract of Garcinia cowa leaves was tested against gram positive and gram negative bacteria. In case of 300 µg/disc, the highest antimicrobial activity was shown against *Pseudomonas aureus*. The diameter of the zone of inhibition was 12mm compared to the 30 mm of diameter of the zone of inhibition of the standard Gentamycin. It showed the moderate activity against *Bacillus subtilis, Salmonella paratyphi, Salmonella typhi, Shigella dysenteriae, Vibrio parahemolyticus*, *Staphylococcus aureus* and *Escherichia coli*. In case of 600 µg/disc, the highest antimicrobial activity was shown against *Bacillus megaterium* and *Sarcina lutea*. The diameter of the zone of inhibition was 12mm compared to the 30 mm of diameter of the zone of inhibition of the standard Gentamycin.

Further investigations are required & researches should focus and explore the specific cellular and molecular targets of various constituents which can develop an unknown medicine for a known fatal disease.

# Conclusion

The results of my study clearly establish the fact that the pet ether extract of possesses both antioxidant activity and antimicrobial activity of *Garicina cowa*.

However, the components responsible for antimicrobial activity of the extracts are clear. Future studies will be aimed at investigating the effects of different parts upon isolating and identifying the substances responsible for the antimicrobial effects of the solvent extracts.

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

# References

Auranwiwat, C., Trisuwan, K., Saiai, A., Pyne, S.G. and Ritthiwigrom, T., (2014). "Antibacterial tetraoxygenated xanthones from the immature fruits of Garcinia cowa. *Fitoterapia*" *98*, pp.179-183.

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

Bregum, F., (2010). "The present status of Medicinal Plants in Bangladesh" *Iranian Journal of Pharmaceutical Research*, pp.34-34.

Dixie, G., Imam, S.A. and Hussain, M.J., (2005). "Medicinal plant marketing in Bangladesh" Intercooperation.

Fabricant, D.S. and Farnsworth, N.R., (2001). "The value of plants used in traditional medicine for drug discovery" *Environmental health perspectives, 109*(Suppl 1), p.69.

Ghani, A., (1998). "*Medicinal plants of Bangladesh: chemical constituents and uses"* Asiatic society of Bangladesh.

Handa, S, Khanuja, S, Longo, G. and Rakesh, D. (2008). "Extraction technologies for medicinal and aromatic plants" *International centre for science and high technology.* [online]

Igho, O., Kang, H.S., Rachel, P., Barbara, W. and Edzard, E., (2010). "The use of Garcinia extract (hydroxycitric acid) as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials" *Journal of obesity*, *2011*.

Jena, B.S., Jayaprakasha, G.K. and Sakariah, K.K., (2002). "Organic acids from leaves, fruits, and rinds of Garcinia cowa" *Journal of Agricultural and Food chemistry*, *50*(12), pp.3431-3434.

Joseph, G.S., Jayaprakasha, G.K., Selvi, A.T., Jena, B.S. and Sakariah, K.K., (2005). "Antiaflatoxigenic and antioxidant activities of Garcinia extracts" *International Journal of Food Microbiology*, *101*(2), pp.153-160.

Jackson, Mark (2011). "The Oxford_ Handbook of the History of Medicine" Oxford University Press. pp.672

Kaennakam, S., Siripong, P. and Tip-pyang, S., (2015). "Kaennacowanols A–C, three new xanthones and their cytotoxicity from the roots of Garcinia cowa. *Fitoterapia*" *102*, pp.171-176.

Kasote, D.M., Katyare, S.S., Hegde, M.V. and Bae, H., (2015). "Significance of antioxidant potential of plants and its relevance to therapeutic applications*" International journal of biological sciences*, 11(8), p.982.

Katiyar, C., Gupta, A., Kanjilal, S. and Katiyar, S., (2012). "Drug discovery from plant sources: An integrated approach" *Ayu*, *33*(1), p.10.

Liu, Y., Chen, J., Bai, Z., Deng, X. and Zhang, L., (2002). "Seed dispersal, seed predation, and seedling spatial pattern of Garcinia cowa (Guttiferae)" *Acta Phytoecological Sinica*, *26*(4), pp.427-434.

Negi, P.S., Jayaprakasha, G.K. and Jena, B.S., (2008). "Antibacterial activity of the extracts from the fruit rinds of Garcinia cowa and Garcinia pedunculata against food borne pathogens and spoilage bacteria" *LWT-Food Science and Technology*, *41*(10), pp.1857-1861.

Pillai, D.S., Prabhasankar, P., Jena, B.S. and Anandharamakrishnan, C., (2012). "Microencapsulation of Garcinia cowa fruit extract and effect of its use on pasta process and quality" *International Journal of Food Properties*, *15*(3), pp.590-604.

Panthong, K., Hutadilok-Towatana, N. and Panthong, A., (2009). "Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from Garcinia cowa" *Canadian Journal of Chemistry*, *87*(11), pp.1636-1640.

Reller, L.B., Weinstein, M., Jorgensen, J.H. and Ferraro, M.J., (2009). "Antimicrobial susceptibility testing: a review of general principles and contemporary practices*" Clinical infectious diseases, 49*(11), pp.1749-1755.

Rates, S.M.K., (2001). "Plants as source of drugs" *Toxicon, 39*(5), pp.603-613.

Rahman, M.M., Islam, M.B., Biswas, M. and Alam, A.K., (2015). "In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh*" BMC research notes,* 8(1), p.621.

Sofowora A. (1982). "Medicinal Plants & Traditional Medicine in Africa. Chichester"

Sitesh C Bachar. (2013). "Medicinal plants in Bangladesh" Available from: http://pharmabiz.com/PrintArticle.aspx?aid=77992&sid=0

Sharma, A., Joseph, G.S. and Singh, R.P., (2014). "Antioxidant and antiplatlet aggregation properties of bark extracts of Garcinia pedunculata and Garcinia cowa" *Journal of food science and technology*, *51*(8), pp.1626-1631.

Sriyatep, T., Siridechakorn, I., Maneerat, W., Pansanit, A., Ritthiwigrom, T., Andersen, R.J. and Laphookhieo, S., (2015). "Bioactive prenylated xanthones from the young fruits and flowers of Garcinia cowa" *Journal of natural products*, *78*(2), pp.265-271.

Shen, J. and Yang, J.S., (2006). "Two new xanthones from the stems of Garcinia cowa" *Chemical and pharmaceutical bulletin*, *54*(1), pp.126-128.

Schimmel, K.J., Richel, D.J., van den Brink, R.B. and Guchelaar, H.J., (2004). "Cardiotoxicity of cytotoxic drugs" *Cancer treatment reviews*, *30*(2), pp.181-191.

Sharmin, T., Chowdhury, S.R., Mian, M.Y., Hoque, M., Sumsujjaman, M. and Nahar, F., (2014). "Evaluation of antimicrobial activities of some Bangladeshi medicinal plants" *World J Pharm Sci*, *2*(2), pp.170-175.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha, L.Y., (2011). "Extraction, isolation and characterization of bioactive compounds from plants extracts*" African Journal of Traditional, Complementary and Alternative Medicines* pp.*8*(1).

Wahyuni, F.S., Arisanty, D., Hayaty, N.F. and Juwita, D.A., (2017). "Sub-acute Toxicity Study of The Ethyl Acetate Fraction of Asam Kandis Rinds (Garcinia cowa Roxb.) on the Liver and Renal Function in Mice" *Pharmacognosy Journal*, *9*(3).

Williamson, E.M., Okpako, D.T. and Evans, F.J., (1996). "Pharmacological methods in phytotherapy research: volume 1: Selection, preparation and pharmacological evaluation of plant material" John Wiley & Sons Ltd.

Wahyuni, F.S., Shaari, K., Stanslas, J., Lajis, N.H. and Hamidi, D., (2016). "Cytotoxic properties and complete nuclear magnetic resonance assignment of isolated xanthones from the root of Garcinia cowa Roxb" *Pharmacognosy magazine*, *12*(Suppl 1), pp.S52.

Wadood, A., Ghufran, M., Jamal, S.B., Naeem, M., Khan, A. and Ghaffar, R., (2013). "Phytochemical analysis of medicinal plants occurring in local area of Mardan" *Biochem Anal Biochem*, *2*(4), pp.1-4.

Wahyuni, F.S., Shaari, K., Stanslas, J., Lajis, N. and Hamidi, D., (2015). "Cytotoxic compounds from the leaves of Garcinia cowa Roxb"