Antioxidant and Antimicrobial Investigations of dichloromethane(DCM) Extract of *Garcinia cowa* Bark

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

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DECLARATION BY THE RESEARCH CANDIDADE

I,Razuana Bashir Mousume, hereby declare that this dissertation, entitled '**Antioxidant and Antimicrobial Investigations of dichloromethane (DCM) Extract of *Garcinia cowa* Bark'** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma or Fellowship.

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled '**Antioxidant and Antimicrobial Investigations of dichloromethane (DCM) Extract of *Garcinia cowa* Bark**' is a research work carried out by Razuana Bashir Mousume (ID: 2013-3-70-035) in 2017, under the supervision and guidance of me, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled is a research work carried out '**Antioxidant and Antimicrobial Investigations of Dichloromethane (DCM) Extract of *Garcinia cowa* Bark**' by Razuana Bashir Mousume (ID: 2013-3-70-035), under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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ABSTRACT

Many plants of the genus *Garcinia* (Family Clusiaceae) have been used in traditional medicine in several parts of the world for treatment of the most different illnesses. In Thailand, where twenty-nine species have been observed, *G. mangostana*, *G. speciosa* and *G. cowa* have been widely used in Thai folk medicine. The aim of the present study was to evaluate the antioxidant and antimicrobial activity of DCM extract of *Garcinia cowa*.The antioxidant activity was measured by DPPH scavenging assay and total phenol content.The IC_{50} values of DPPH scavenging assay was 69.903 µg/ml for DCM extract of *Garcinia cowa* bark. The total phenol content was 362.93±18 mg/g equivalent to gallic acid for DCM extract of *Garcinia cowa* bark. By determining antioxidant property the present result suggest that the tested plant extract have potent antioxidant activity. The anti microbial activity of DCM extract of *Garcinia cowa* plant were tested against ten microorganism by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The DCM extract of *Garcinia cowa* plant showed might ((7mm-8mm) antimicrobial activities against the microorganism. The DCM extract of *Garcinia cowa* showed highest activity against *vibrio parahemolyticus,Salmonella typhi,Bacillus sereus* and moderate activity against *Bacillus subitilis.* No activity against was observed *E.coli, Salmonella paratyphi, Staphylococcus aureus, pseudomonas aureus*. Further investigations needed to identify the active constituents and the exact mechanism of action responsible for reported antioxidant and antimicrobial properties of *Garcinia cowa.*

Key words : *Garcinia cowa,* Antimicrobial activities, Antioxidant activities, DCM extract of *Garcinia cowa* bark, DPPH scavenging assay, Total phenol content.

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Chapter-1

INTRODUCTION

1.1 Introduction

The term "**medicinal plant**" include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.

The word "**herb**" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now a days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda,

Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure.

Medicinal plants such as *Aloe, Tulsi, Neem, Turmeric* and *Ginger* cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (*Tulsi*) for making medicines, black tea, in *pooja* and other activities in their day to day life.

In several parts of the world many herbs are used to ***honou**r* their kings showing it as a symbol of luck. Now, after finding the role of herbs in medicine, lots of consumers started the plantation of tulsi and other medicinal plants in their home gardens.

Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.

Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Now a days medicinal herbs are important sources for pharmaceutical manufacturing.

Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively.

Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO

has published three volumes of the WHO monographs on selected medicinal plants (Lichterman,B.L, 2004).

1.2 Medicinal Plant:

Background

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found. One of these allegedly ancient medicinal herbs, yarrow, is discussed in this work as a modern medicinal plant. Since prehistoric times, shamans or medicine men and women of Eurasia and the Americas acquired a tremendous knowledge of medicinal plants. All of the native plant species discussed in detail in this work was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations Canadians (Arnason et al. 1981) suggests that many of these also have important pharmacological constituents that could be valuable in modern medicine. Up until the 18th century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16th century Italy, in Pisa, Padova and Florence, were medicinal plant gardens attached to medical faculties or schools. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke 1985). A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients (Duke 1993).

1.2.1 The Definition of Medicinal Plants

This category includes all plants any or all parts of which are used for therapeutical purposes due to the active ingredients contained in them. They can be wild plants or cultivated ones. Since cultivated plants have numerous beneficial effects too, in a larger sense, any plant can be a

medicinal herb, including arable plants, vegetables, fruits, and spices. Presumably many of them had been originally used as medicinal herbs in preserving food or treating gastric disorders, and became spices because of their beneficial effects, pleasant smell and taste. Addictive substances such as caffeine also have curative effects therefore their consumption in therapeutical doses falls into a different category.

The categorization of plants – into arable plants, ornamentals, poisonous plants, weeds, etc. – is always subjective; there is always a human element in it and reflects a certain attitude, economic interests, a purpose or a goal, etc. A plant can belong to several categories, depending on which of its characteristics is emphasized.

The above quoted ancient story from the history of Indian therapeutics, in which the studies of Jivaka ended when he could not find a single plant with no beneficial effects after several days of searching, is very relevant and expressive. But our job, besides broadening the selection, is to direct attention to easily obtainable and more effective herbs.

In the history of herbal medicine, there have been extreme views too. For example, it was held that every plant is effective against every disease, which is apparently a wild exaggeration. But it is certainly important to use those plants as medicinal herbs which according to our knowledge and experience have the strongest effects coupled with the least (or no) side effects. We also have to consider that similar to pharmaceuticals, medicinal herbs do not affect everyone in the same way. Depending on the individual's reaction, various herbs can be indicated as the best remedy (Grene, Marjorie,2004).

1.2.2 Medicinal Uses of Pimpinella Anisum

- The properties of PimpinellaAnisum make it a natural choice to alleviate flatulence.
- When mixed with other oils, Aniseed oil provides a good antiseptic.
- It is of great help for providing relief when suffering from a dry *cough* used as a common cure in cases of infant catarrh.
- Used externally for treating lice infestation and scabies.
- Used commonly in mouthwash

Uses of neem

- Antibacterial Potential.
- Detoxifying Effects:
- Gastric Health:
- Cancer and Chronic Disease
- Fungal Infections
- Diabetes treatment
- Malaria Treatment

1.3 Role of Plants In Human History

Plants have also been used in the production of stimulant beverages (e.g. tea, coffee, cocoa, and cola) and inebriants or intoxicants (e.g., wine, beer, and kava) in many cultures since ancient times, and this trend continues till today. Tea (*Thea sinensis*) was first consumed in ancient China (the earliest reference is around CE 350), while coffee (*Coffeaarabica*) was initially cultivated in Yemen for commercial purposes in the 9th century. The Aztec nobility used to consume bitter beverages containing raw cocoa beans (*Theobroma cacao*), red peppers, and various herbs. Nowadays, tea, coffee, and cocoa are important commodities and their consumption has spread worldwide. The active components of these stimulants are methylated xanthine derivatives, namely caffeine, theophylline, and theobromine, which are the main constituents of coffee, tea, and cocoa, respectively. The most popular inebriants in society today are wine, beer, and liquor made from the fermentation of fruits and cereals. Wine was first fermented about 6000–8000 years ago in the Middle East, while the first beer was brewed around 5000–6000 BCE by the Babylonians. The intoxicating ingredient of these drinks is ethanol, a by-product of bacterial fermentation, rather than secondary plant metabolites. Recent studies have shown that a low to moderate consumption of red wine is associated with reduction of mortality due to cardiovascular disease and cancer (Susan et al, 2013).

1.4 The Value of Plants In Our Lives

Ancient Man is known to have utilized plants as drugs for millennia. Based on current knowledge, at least in the West, we know that extracts of some of these plants are useful in a crude form, i.e. Atropa belladonna Tincture as an antispasmodic, Rauvolfiaserpentina roots for hypertension and as a tranquilizer, Papaver somniferum extract or tincture as an analgesic, etc. Further, we know that at least 121 chemical substances of known structure are still extracted from plants that are useful as drugs throughout the world. A large number of plants are used in traditional medical practices, and have been for more than 3000 years, such as in Chinese Traditional Medicine, Indian Traditional Medicine, etc., most of which probably exert therapeutic effects and would be proven as such if they were properly evaluated by Western standards. Still further, plants have been employed for centuries by primitive cultures; most of these are less likely to pass the test of modern experimental verification of efficacy. Finally, there are a large number of so-called herbal remedies, mainly sold in health food stores in developed countries, many of which remain to be verified for their real therapeutic effects. Several years ago the World Health Organization made an attempt to identify all medicinal plants that exist in the world. It was admitted that the compilation of names of medicinal plants undoubtedly contained many replicates since botanical verification was not attempted. Further, the list including more than 20,000 species only provided Latin binomials and the countries where the plants were used, but excluded data indicating what the plants were used for(Margulis, L. ,1974).

1.5 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 (John, 2002). 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 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals (Robson and Beak, 2009). 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 (Hong and Francis, 2004).

Figure 1.1 : Great Moments in Medicine

people (Balick and Cox, 1996).Traditional use of medicine is recognized as a way to learn about potential futuremedicines. 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 and Farnsworth, 2001).

Figure 1.2 : Medicine in Ancient Times

1.6 Importance of some herbs with their medicinal values

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.
- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.
- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.
- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.

- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as *Chirayta*, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.
- Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.
- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.
- Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.
- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.
- Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.
- Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.
- Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.
- Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.

- Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.
- Certain aromatic plants such as Aloe, Golden seal, Barberry and Chirayata are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.
- Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne (Lal Mirch, Myrrh, Camphor and Guggul.
- A wide variety of herbs including Giloe, Golden seal, Aloe and Barberry are used as tonics. They can also be nutritive and rejuvenate a healthy as well as diseased individual.
- Honey, turmeric, marshmallow and liquorice can effectively treat a fresh cut and wound. They are termed as vulnerary herbs.

Plant	**Common Name and Family**	**Parts Used**	**Medicinal Use**
	Amla Family:Euphorbiaceac	Fruit	Vitamin C, cough,Diabetes,cold,Laxitive ,hyperacidity
	Ashok Family: Caesalpinanceac	Bark,flower	Menstrual,Pain,Uterine,Diabetes
	Aswagandha Family:Solanaceac	Root, leafs	Restorative tonic,Stress,Nerves disorder,Aphrodiasiac
	Bael Family:Rutaceac	Fruit,Bark	Diarrhoea,Dysentery
	Bhumi Amla Family: Euphorbiaceac	Whole plant	Aenemic,Jaundice,Dropsy

	Brahmi Family: scrophulariaceac	Whole plant	Nervous memory,Mental disorder
	Kalmegh Family:Scanthaaceac	Whole plant	Fever,Weekness,Release of gas
	Long peeper Family:Piperaceac	Fruit,Roots	Bronchitis,Cold,Antidot
	Makoi,kakamachi Family: Solanaceac	Fruit,whole plant	Diuretic,Anti-dysenteric
	Pashan,Bheda Family: Lamiaceac	Root	Stone
	Sandal Wood Family:Santalinaceac	Wood	Skin disorder,Burning sensation
	Sarpa Ghandha Family:Apocynaceac	Root	Hypertension
	Satavari Family: Liliaceac	Root	Fatigue,Cough
	Senna Family: Liliaceac	Dry tubers	Tonic,Aphrodisiac

	Tulsi Family:Lamiaceac	Leaves	Cough,cold,Bronchitis
	Vai vidanka Family: Myrsinaceac	Leaves , fruit	Skin disease,Snike bite
	Pippermint perennial Family: Lamiaceac	Leaves, Flower	Digestive,Pain killer
	Henna,Mehedi Family: Lytharaceac	Leaf,Flower	Anti-inflammatory
	Gritkumari Family: Liliaceac	Leaves	Laxative,Wound,Skin burn
	Sada Bahar Family:Apocyanaceac	Whole plant	Leaukamia,Anti-spasmodic
	Vringraj Family: Compositae	Whole Powder	Anti-inflammatory,Digestive
	Rakta chitrak Family:Plumbaginaceac	Root	Inflammation,Cough
	Kochila Family:Loganiaceac	Seed	Nervous,Paralysis,Wound

	Harida Family: Combretaceac	powder	Ulcer,Leprosy,Cough
	Bahada	Seed,Bark,Fruit	Insomnia,Vomiting
	Neem Family: Mahaceac	Rhizome	Urinary,Sedative,Hypertensive
	Bach Family: Araceac	Rhizome	Sedative,Analgesic,Hypertensive
	Nageswar champa Family:Guttiferae	Bark,Leaf,Flower	Asthma,Skin burning,Vomiting,Piles
	Kurai Family: Apocyanaceac	Bark seed	Anti-pyretic,Dysentery
	Dalchini Family: Lauraceac	Bark oil	Bronchitis,Asthma,Cardiac disorder,Fever

Table 1.1: List of important medicinal plants and their usages

1.7 The Medicinal Plants contribution in the New World

Just Before Modern Medicine: At the early of modern medicine the Muslim physicians were done a great job. The Arabian Muslim physicians, like Al-Razi and IbnSina (9th to 12th century

AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi‟s important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah, Maan La YahoduruhoTibb etc.The famous medical book, Al-Kanun, of IbnSina was the prescribed book of medicine in the schools of western medicine for several centuries (Mian&Ghani., 1990). The use of medicinal plants in Europe in the 13th and 14th centuries was based on the Doctrine of Signatures or Similar developed by Paracelsus (1490-1541 AD), aswiss alchemist and physician (Murray, 1995). The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca (Erythroxylum species) and tobacco (Nicotianatabacum) was common in these countries in the 14th and 15th centuries. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the Rig Veda (4500-1600 BC). It supplies various information of the medicinal use of plants in the Indian subcontinent (Hill, 1972). Medicinal plants used by the Australian aborigines many centuries ago tremendously enriched the stock of medicinal plants of the world. The current list of the medicinal plants growing around the world includes more than a thousand items (Sofowora, A., 1982).

1.8 Importance of Medicinal Plant

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

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

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

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

Commercial value: growing appreciation of trade and other commercial economic opportunities represented by medicinal plants.

However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine.

In parts of Europe, especially in Germany, herbal medicine (or phytomedicine) is much more popular than is the case in North America. Some 67,000 different herbal products are available in Germany. The already well-established medicinal plant trade of Europe is increasing at an annual rate of about 10%. In Canada, and the US, the regulatory climate has been much less receptive to herbal medicines. This is because lack of proper scientific evaluation, limited regulation, absence of quality control, limited education of many herbal practitioners, and the presence of "snake-oil salesmen" have all combined to give herbal medicine a bad reputation. However, in response to public demand for "alternative" or "complementary" medicine, this situation is changing. At least 20% of Canadians have used some form of alternative therapy, such as herbalism, naturopathy, acupuncture, and homeopathy. Herbs are the fastest-growing part of the pharmacy industry of North America, with an annual growth variously estimated as 15 to 20%, and thousands of herbal products are now available to Canadians. Herbal remedies have been estimated to have a current value of between two and ten billion dollars in North America, depending on how comprehensively the category of medicinal herbs is interpreted predicted that with appropriate research and regulation, "herbal medicine will regain its rightful status as an important and integral aspect of classical medicine"(Balick and Cox, 1996).

1.9 Ten Interesting Facts About Complementary and Alternative Medicine

1. The World Health Organization estimates that between 65 to 80 percent of the world‘s population (over 4 billion people) rely on alternative medicine as their primary form of health care compared to only 10 to 30 percent of people who use conventional medicine.

2. Traditional Chinese medicine has been chosen by the World Health Organization for worldwide propagation to meet the heath care needs of the twenty-first century.

3. Medicinal herbs were found amongst the personal effects of the mummified prehistoric ―ice man who was found in the Italian Alps in 1991.

4. 19 percent of Fortune 500 companies offer alternative medicine as part of their health care compensation packages.

5. One-half of all medical schools now offer courses in alternative medicine.

6. Spinal manipulation was used by the Ancient Greeks long before it was incorporated into chiropractic and osteopathic medicine in the 19th Century.

7. More than 70% to 90% of physicians consider complementary and alternative medicine therapies, such as diet and exercise, behavioral medicine, counseling and psychotherapy, and hypnotherapy, to be legitimate medical practices.

8. Massage therapy dates back thousands of years and has been recorded in ancient writings from the Orient, Asia, Arabia and Greece.

9. The National Institutes of Health currently invests about $40 million per year in complementary and alternative medicine related research.

10. 2/3 of people who use complementary and alternative medicine do not tell their medical doctor.

Many of the chemicals and medicines that have been developed over the years are in fact based on active ingredients present in herbs and plants (Drey,2013).

1.10 Classification of Medicinal Plant

Classification of medicinal plants is organized in different ways depending on the criteria used. In general, medicinal plants are arranged according to their active principles in their storage organs of plants, particularly roots, leaves, flowers, seeds and other parts of plant. These principles are valuable to mankind in the treatment of diseases. Reports on the classification of many plant species yielding vegetable oils used in cosmetics and body and skin care preparations are sporadic or lacking. Herbs are classified in many ways. Some of them are:

1. according to the usage;

2. according to the active constituents;

3. according to the period of life;

4. according to their taxonomy;

5. according to their habitats

1.10.1 Classification According to the Usage

The herbs are classified in four parts: medicinal herbs, culinary herbs, aromatic herbs, ornamental herbs.

A. Medicinal Herbs have curative powers and are used in making medicines because of their healing properties like marigold, lemon balm, lavender, johnny-jump-up feverfewetc.
B. Culinary Herbs are probably the mostly used as cooking herbs because of their strong flavourslike oregano, parsley, sweet basil, horseradish, thyme etc.
C. Aromatic Herbs have some common uses because of their pleasant smelling flowers or foliage. Oils from aromatic herbs can be used to produce perfumes, toilet water, and various scents For e.g. mint, rosemary, basil etc
D. Ornamental Herbs are used for decoration because they have brightly coloured flowers and foliage like lavender, chives, bee balm, lemongrass etc.

1.11 Global Scenario of Medicinal Plants

According to the World Health Organization (WHO), more than 80% of the world's of population relies on traditional medicine for their primary health care needs. The use herbal medicines in Asia represents a long history of human interactions with the environment. Herbal medicine is a common element in Ayurvedic, homeopathic,natu uropathic, traditional and oriental, Native American & 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.worth about US$ 62 billion per annum.Annual growth of herbal market is about 15percent and the global herbal market by 2050 is expected to be about US$ 5 trillion(Payyappallimana, 2009).Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide (Rates,2001).

1.12 Antioxidant:

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

Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid et al. 2010). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia,

Alzheimer's disease, infl tory disease, muscular dystrophy, liver disorder, and even aging (Amit and Priyadarsini 2011). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet (Teresa et al. 2011).

1.12.1 Classification of Antioxidants

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

1.12.2 Natural Antioxidants

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

1.12.3 Enzymatic Antioxidants

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

1.12.4 Primary Antioxidants

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

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

1.12.5 Secondary Antioxidant

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

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

1.12.6 Nonenzymatic Antioxidants

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

1.12.6.1 Carotenoid

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

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

1.12.6.2 Polyphenols

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

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

1.12.7 Other Antioxidants

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

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

1.13 Plants under the *Garcinia* Class:

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

mangostana), garcinias or, ambiguously, "monkey fruit".Many species are threatened by habitat destruction, and at least *G. cadelliana* from South Andaman Island is almost or even completely extinct already. The fruits are a food source for several animals, such as the archduke butterflies (Lexias) of tropical eastern Asia which relish the sap of overripe mangosteens. Garcinia species are evergreen trees and shrubs, dioecious and in several cases apomictic. The fruit is a berry with fleshy endocarp, which in several species is delicious.

1.13.1 Different species of *Garcinia*:

Some selected species are :

- *Garcinia acutifolia*
- *Garcinia afzelii*
- *Garcinia aristata*
- *Garcinia atroviridis* – *asam gelugur* (Indonesian), *asam gelugor* (Malaysian), *asam keping* (Malaysian)
- *Garcinia benthami*
- *Garcinia bifasciculata*
- *Garcinia brassii*
- *Garcinia brevipedicellata*
- *Garcinia burkillii*
- *Garcinia cadelliana*
- *Garcinia cambogia*
- *Garcinia cantleyana*
- *Garcinia cerasifer* (H.Perrier) P.F.Stevens
- *Garcinia clusiaefolia*
- *Garcinia costata*
- *Garcinia cymosa* (K.Schum.) I.M.Turner & P.F.Stevens
- *Garcinia decussata*
- *Garcinia diversifolia*
- *Garcinia dulcis* – *mundu, rata*

- *Garcinia echinocarpa*
- *Garcinia elliptica*
- *Garcinia epunctata*
- *Garcinia eugeniaefolia*
- *Garcinia forbesii*
- *Garcinia fragraeoides*
- *Garcinia gardneriana* – *bacupari*

Figure 1.3 : Habitat of Garcinia Class Plants Around the World

Figure1.4: ***Garcinia cowa*** tree in Bangladesh

1.13.2 Use of *Garcinia* Class:

The fruit of most species of *Garcinia* are eaten locally; some species fruits are highly esteemed in one region, but unknown just a few hundred kilometres away. The best-known species is the purple mangosteen (*G. mangostana*), which is now cultivated throughout Southeast Asia and other tropical countries, having become established in the late 20th century. Less well-known, but still of international importance, are kandis (*G. forbesii*) with small round red fruits with subacid taste and melting flesh, the lemon drop mangosteen (*G. intermedia*) with yellow fruit that look like a wrinkled lemon, and the thin-skinned orange button mangosteen (*G. prainiana*).In addition, mangosteen rind (exocarp) extract is used as a spice. It figures prominently in Kodava culture, and *G. multiflora* is used to flavour and colour the famous *bún riêu* soup of Vietnam, where this plant is known as *hạt điều màu*. *Garcinia gummi-gutta* yields a spice widely used in South Asia, in particular in Kerala, where it is called *kodumpulli*.Most species in *Garcinia* are known for their gum resin, brownish-yellow from xanthonoids such as mangostin, and used as purgative or cathartic, but most frequently – at least in former times – as a pigment. The colour term gamboge refers to this pigment. Extracts of the exocarp of certain species – typically *G. gummi-gutta*, but also purple mangosteen – are often contained in appetite suppressants such as Hydroxycut, Leptoprin or XanGo. But their effectiveness at normal

consumption levels is unproven, while at least one case of severe acidosis caused by long-term consumption of such products has been documented. Furthermore, they may contain significant amounts of hydroxycitric acid, which is somewhat toxic and might even destroy the testicles after prolonged use. Bitter kola (*G. kola*) seeds are used in folk medicine. *G. mannii* is popular as a chew stick in western Africa, freshening the breath and cleaning the teeth.*G.subelliptica*,called *fukugi* in Japanese,isthe floralemblem of Mobuto and Tarama on Okin awa. The Malaysian town of Beruas – often spelled "Bruas" – derives its name from the seashore mangosteen (*G. hombroniana*), known locally as *pokok bruas*(WONG, L.P,2008).

1.14 Garcinia Cowa Plant Description

1.14.1 Description of plant

Family: Clusiaceae

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

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

English name: Cow Tree

A medium-sized evergreen tree with horizontal branches and oval crown. Leaves 7.6-12.6 cm long, broadly to elliptically lanceolate, acuminate. Flower rather small, yellow; the male ones smaller in dense terminal clusters; the females 13 mm diam., or somewhat larger, solitary or by 3-5 at the end of the branchlets. Berry the size of a lime, slightly 6-8 lobed, dul red, somewhat depressed at the apex.

1.14.2 Using Information:

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).

1.14.3 Chemical constituents:

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

Figure1.5: Parts of *G. cowa*: (a) branch, (b) bark and latex, (c) inflorescences, (d) leaves and (e) ripe and immature fruits (Photos taken by S. Laphookhieo, 2011).

The genus Garcinia has over 200 species distributed in the tropics of the world. About 35 species occur in India, many of which are endemic and economically important with immense medicinal properties. However, lack of awareness, coupled with habitat destruction, leads to genetic erosion of this forest resource and many species are threatened. The Indian Institute of Spices

Research (IISR), Calicut, has Garcinia genetic resources' collection of 15 species of Western Ghats and Eastern Himalaya species. The morphological characterisation of the species of these two different eco systems indicates that there are variations within the species of the same ecosystem while there are similarities in the species of two different ecosystems.The genus Garcinia (Family: Clusiaceae) consists of over 200 species distributed in the tropics of the world chiefly in Asia, Africa, and Polynesia. They are evergreen polygamous trees, shrubs, and herbs. About 35 species are reported to exist in India, many of which are endemic and economically important with immense medicinal properties.In India, species of Garcinia grow extensively in semiwild state, in the Konkan region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, and evergreen forests of Assam, Khasi, Jantia hills, Nagaland, West Bengal, and Gujarat. In Malabar and Konkan regions of Southern India, they are used in garnishing curries and also as a replacement for tamarind. In North Eastern India, the sundried slices of the fruits are used for culinary purposes and as folk medicine. Some species like Garcinia cambogia, G. indica, and G. cowa are cultivated in certain parts of India. G. pedunculata, G. kydia, G. cowa, and G.lanceaefolia are the most important species in north eastern parts of India. Many species of Garcinia have fruit with edible arils and are eaten locally. The best-known species is the mangosteen (G. mangostana), which is now cultivated throughout Southeast Asia and other tropical countries. The seeds of G. indica fruits yield valuable edible fat known as kokum butter. The fruits of Garcinia are a food source for several animals. Most species in Garcinia are known for their gum resin which is used as purgative or cathartic. Fruits of some Garcinia species are also one of the richest sources of red pigments in the plant kingdom. Fruit and syrup of G. indica are very popular in Konkan region and are antioxidant and antibacterial.Garcinia is the source for a natural diet ingredient (-) hydroxycitric acid. HCA (1,2dihydroxypropane-1,2,3-tricarboxylic acid) which is an antiobesity compound is present in the fruit rind and leaves of Garcinia and is known to inhibit lipid and fatty acid synthesis in living systems. HCA is also a hypocholesterolemi agent. On a dry weight basis, HCA constitutes about 20–30% of the fruit. Lack of knowledge, coupled with habitat destruction, leads to genetic erosion of this forest resource and many species are threatened . They need to be studied and conserved. In this study, we tried to characterize the morphological features of the species collected from Western Ghats

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

1.14.4 Methodology

The morphological features of the Indian *Garcinia* were observed for mature and bearing trees.A total of 9 species were studied here from two distinct geographical locations, namely, Western Ghats and Northeast Himalayan foothills.Species selected for this study were *G. indica*, *G. gummi-gutta, G. cowa, G. subelliptica and G. mangostana* from Western Ghats*; G. pedunculata, G. lanceaefolia, and G. kydia* from NE Himalayas*; and G. xanthochymus* from both the *ecosystems.G. gummi-gutta* is also known as *G. cambogia and G. xanthochymus as G. tinctoria. Garcinia cowa and G. kydia* are considered as synonymous though they show some variations in their morphological features.The plant height was calculated by fixed angle of elevation method, in which the distance from the tree is measured, at which the top of the tree coincides with the tip of the right triangle making an angle of 45° with horizontal. The tree height is calculated by adding the distance between tree and observer to the height of the observer to the position of stretched hands.Dimensions of the plant leaves were measured by placing them on a standard linear graph sheet with each divisions of 1 cm and subdivisions of 1 mm. Fruit and seed sizes were measured using a Vernier caliper. The morphological features of plant leaf and flowers were observed and compared with available literatures.

1.15 Spatial Distribution and Interspecific Associations of Tree Species in a Tropical Seasonal Rain Forest of China

Studying the spatial pattern and interspecific associations of plant species may provide valuable insights into processes and mechanisms that maintain species coexistence. Point pattern analysis was used to analyze the spatial distribution patterns of twenty dominant tree species, their interspecific spatial associations and changes across life stages in a 20-ha permanent plot of seasonal tropical rainforest in Xishuangbanna, China, to test mechanisms maintaining species coexistence. Torus- translation tests were used to quantify positive or negative associations of the species to topographic habitats. The results showed: (1) fourteen of the twenty tree species were negatively (or positively) associated with one or two of the topographic variables, which evidences that the niche contributes to the spatial pattern of these species. (2) Most saplings of the study species showed a significantly clumped distribution at small scales (0–10 m) which was lost at larger scales (10– 30 m). (3) The degree of spatial clumping deceases from saplings, to poles, to adults indicates that density-dependent mortality of the offspring is ubiquitous in species. (4) It is notable that a high number of positive small-scale interactions were found among the twenty species. For saplings, 42.6% of all combinations of species pairs showed positive associations at neighborhood scales up to five meters, but only 38.4% were negative. For poles and adults, positive associations at these distances still made up 45.5% and 29.5%, respectively. In conclusion, there is considerable evidence for the presence of positive interactions among the tree species, which suggests that species herd protection may occur in our plot. In addition, niche assembly and limited dispersal (likely) contribute to the spatial patterns of tree species in the tropical seasonal rain forest in Xishuangbanna, China.

2.1 Dormancy Breaking and Storage Behavior of *Garcinia cowa* Roxb. (Guttiferae) Seeds: Implications for Ecological Function and Germplasm Conservation

The dormancy breaking and storage behavior of *Garcinia cowa* Roxb. seeds were investigated.The seeds of *G. cowa* had 8–11 months dormancy in their natural habitat. Seeds were matured and dispersed at the end of the rainy season (mid-late August to late September) and were scatter-hoarded by rodents as food for winter after the seeds had fallen to the ground. Seedlings often emerged in the forest during the rainy season (May to August) the following year. Intact seeds of *G. cowa* failed to germinate after being sown at 30 °C for 120 d and the mean germination time (MGT) of seeds cultured in a shade (50% sunlight) nursery was 252 d. The most effective method of breaking dormancy was to remove the seed coat totally,which reduced the MGT to 13 d at 30 °C. Germination was also promoted by partial removal of the seed coat(excising the hilum and exposing the radicle) and chemical scarification (immersion in 1% H2O2 for 1 d).Unscarified seeds take up water rapidly in the first 96 h, but water was absorbed by the outside seed coat,without penetrating through it. The moisture content (MC) of *G. cowa* seeds was high (50% in fresh weight)at shedding. The seeds could tolerate desiccation to some extent, until the MC reached approximately 40%;below that, the viability decreases rapidly and all seeds died at approximately 17% of MC. Seed viability decreased rapidly when seeds were chilled at 4 °C; germination was 2% after storage for 1 week. Even stored at 10 °C, seeds began to be damaged after 4 weeks. Seed storage for 1 yr revealed that in both dry (relative humidity (35 ± 5)%) and moist (wet sand) storage conditions, seed viability declined, but germination

percentages for seeds stored under moist conditions are better than for seed stored under dry conditions.Because of their low tolerance to desiccation, marked chilling sensitivity and

relatively short lifespan, *G.cowa* seeds should be classified into the tropical recalcitrant category. The ecological implications of dormant recalcitrant seeds and cues on storing recalcitrant seeds were discussed(Yoda, Kira, et al. – 1963).

2.2 Cytotoxic Compounds from the Leaves of *Garcinia Cowa* Roxb.

The Genus *Garcinia*, belonging to the Family Clusiaceae have been widely investigated in terms of their bioactive ingredients. The plants are small to medium sized trees,which grow up to 30 m in height and are widely distributed in the tropical regions of the world (Kijjoa and Vieira, 2009). This genus has various biological activities such as antioxidant (Muharni *et al.*, 2009 and Dachriyanus *et al.*, 2003), cytotoxic (Wahyuni *et al.*, 2009) and antimicrobial activities (Dachriyanus*et al.*, 2004). *Garcinia cowa* Roxb known as asam kandis in West Sumatera It is widely distributed throughout Indonesia and the Malay peninsula. The fruits are edible with a sour taste and used as spices in Indonesia especially in Minang tribes. (Dachriyanus *et al.*, 2003). Many parts of *G. cowa* have been used in traditional folk medicine. The bark, latex and root have been used while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant .Some pharmacological properties such as antitumorpromoting(Mukarami *et al.*, 1995), inhibition of human lowdensity lipoprotein peroxidation and anti-platelet activities have been reported on the crude extract of leaves .The chemical composition and biological activities of various parts of *G. cowa* have been investigated. Previous investigation on the fresh leaves, fruits and dried rinds of *G. cowa* has been investigated and found that (-)-hydroxycitric acid and its lactone constitute the major constituents (Jena *et al.*, 2002). Previously, we reported the isolation of[2*E*,6*E*,10*E*]-(+)-4-hydroxy-3-methyl-5-(3,7,11,15-tetramethyl-2,6,10,14 hexadecatetraenyl-2-cyclohexen-1-one (1), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4- (1,1-dimethyl-2-propenyl)-9*H*- xanthen-9-one (2) and rubraxanthone (3) from the stem bark of this plant. (Wahyuni *et al.*, 2004) In continuation of our study on *Garcinia cowa*, cytotoxic properties of isolated compounds from the leaves of *Garcinia cowa* against cancer cell-lines are reported.

Garcinia cowa is an abundant source of bioactive phytochemicals. Phytochemical investigations of the plant parts indicated that the fruit, twig and stem are the best source of secondary metabolites, providing flavonoids,phloroglucinols and xanthones respectively. Seventyeight of these compounds have been identified from theplant and several have interesting pharmacological activities(Laphookhieo, S.Pyne,S.G 2013).

2.3 Antimal Arial Xanthones from *Garcinia Cowa*

Five xanthones from the bark of Garcinia cowa, namely 7-O-methylgarcinone E (1), cowanin (2), cowanol (3), cowaxanthone (4), and beta-mangostin (5), were found to possess in vitro antimalarial activity against Plasmodium falciparum with IC50 values ranging from 1.50 to 3.00 micrograms/ml. Complete 1H- and 13C-NMR assignments of these compounds are also reported (Wahyuni *et al.*, 2004).

2.4 Anti-Inflammatory Activity of Isolated Compounds from the Stem Bark of *Garcinia Cowa Roxb*

To find the anti inflammatory active compounds from methanol extract of *Garcinia cowa*.Methods: To evaluate the inhibitory activity of isolated compounds on nitric oxide (NO) production, culture media was assayed using Griess reaction. An equal volume of Griess reagent (1% sulphanilamide and 0.1% N-(L-naphthyl)-ethylene diamine dihydrochloride, dissolved in 2.5% H3PO4) was mixed with culture supernatant and color development was measured at 550 nm using a micro plate reader. The amount of nitrite in the culture supernatant was calculated from a standard curve (0–100 μM) of sodium nitrite freshly prepared in deionized water. Percentage of the NO inhibition was calculated by using nitrate level of IFN-γ/LPS-induced group as the control. Results: Isolated compounds, tetraprenyltoluquinone, rubraxanthone and α-mangostin from stem bark of *Garcinia cowa* Roxb were evaluated for their anti-inflammatory activity. Only α-mangostin exhibited strong anti-inflammatory activity with 83.42 % inhibition of NO and without inducing severe cytotoxicity at 50 μM. Rubraxanthone showed weak inhibition of NO with 23.86 % inhibition of NO while maintained 77.32 % of cell viability. TPTQ also showed the strong inhibition of NO with 80.98 % inhibition but unfortunately this compound also induced severe cytotoxicity with 39.62% viability. Conclusion: α-Mangostin

exhibited strong anti-inflammatory activity without inducing severe cytotoxicity at 50 μM. Rubraxanthone showed weak inhibition of NO while Tetraprenyltoluquinone also showed the strong inhibition of NO however this compound also induced severe cytotoxicity.

2.5 Antioxidant Activity of *Garcinia* Species of Assam

The present research work deals with the antioxidant activity of *Garcinia species* e.g. *Garcinia pedunculata* , *Garcinia cowa, Garcinia lanceifolia* and *Garcinia xanthochymus* of Assam. Ascorbic acid content varied from 35.37 mg/100g in *Garcinia xanthochymus* to 88.92mg/100g in *Garcinia pedunculata*. Total phenol content was found highest (2.74 mg/g) in *Garcinia lanceifolia*. However the carotenoid content was maximum (30.34 µg/g) in *Garcinia xanthochymus* and total antioxidant activity in terms of IC50 value was found highest (11.61) in *Garcinia pedunculata*. The results indicate that these *Garcinia species* of Assam can be used as a source of antioxidant (Jantan *et al.,* 2011).

2.6 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 *G. cowa.*

2.7 Antifever Activity of *Garcinia Cowa*

Many parts of G. cowa have been used in traditional folk medicine. For example, the bark, latex and root have been used as an antifever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant . The chemical composition and biological activities of various parts of G. cowa have been investigated. The major compounds found were xanthones and phloroglucinols. However, minor compounds, including depsidones, terpenoids, steroids and flavonoids, were also observed. Currently, 78 compounds have been isolated from the twig , stem , fruit and latex . This review mainly focuses on the chemical structures and biological activities of the phytochemicals isolated from G. cowa and covers the literature up to April 2012(Pathong *et al.*, 2009).

2.8 Distribution and Biological Activity

The biological activities of the extracts from various parts of G. cowa have been investigated, including the hexane and chloroform extracts of the fruit rind and methanol extract of the leaves and twigs.The hexane and chloroform extracts from the fruit rind of G. cowa were tested against four Gram-positive bacteria (Bacillus cereus, B. coagulans, B. subtilis and Staphylococcus aureus) and one Gram-negative bacterium (Escherichia coli). Both extracts significantly inhibited bacterial, but not E. coli (IC_{50}s 250-500 μgrowth of the Gram-positive bacteria (IC_{50}s 15-30). The extracts were also found to inhibit the growth of Aspergillus flavus ATCC 46283, a common fungal food contaminant which produces aflatoxin B1. The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (ca 40-60% at the same concentration) . The methanol extracts of the leaves and twigs of G. cowa were evaluated for their ability to inhibit low-density lipoprotein g/mL and wasμperoxidation induced by copper ions. The twig extract had an IC50 value of 20.5 g/mL) than the leaf extract (IC50 not measured). Theμmore potent (higher % inhibition at 1000 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. The structural types, chemical structures and biological activities of the natural products isolated from different parts of *G. cowa(*Maejo Int. J. Sci. Techol. 2013).

2.9 Anti Hyperlipidemia

The aim of this study was to investigate the chemical constituents and in vitro anti-hyperlipidemic activity of *Garcinia cowa* Roxb. ex DC. leaves (Family: Guttiferae). The ethanolic extract of *G. cowa* leaves inhibited cholesterol absoption in Caco-2 and HMG CoA reductase with a percentage inhibition of 14.6 and 97.06 percent, respectively. It showed pancreatic lipase inhibitory effect at the IC_{50}value of 196.60 μg/ml. Further partition of the ethanolic extract of *G. cowa* leaves yielded hexane extract, dichloromethane extract, butanol extract and water extract. The study revealed an interesting hypolipidemic effect of *G. cowa* leaf extracts. At the concentration of 100 μg/ml, the hexane extract and dichloromethane extract inhibited cholesterol absoption in Caco-2 at 36.74 and 32.80 percent inhibition, respectively. At the concentration of 10 μg/ml, the hexane extract and the dichloromethane extract also inhibited HMG CoA reductase at 114.34 and 80.55 percent, respectively. The hexane and dichloromethane extract of *G. cowa* leaves exhibited pancreatic lipase inhibitory activity at the IC_{50}values of 67.45 and 342.80 percent, respectively. Phytochemical study of the dichloromethane extract of *G. cowa* leaves, using chromatographic techniques and structural determination of isolated compounds by means of comparison of the NMR spectral data reported previously, showed that the dichloromethane extract of *G. cowa* leaves consisted of two flavonoid C-glycosides, including vitexin and orientin, and β-sitosterol.

2.10 Antioxidant and Antimutagenic

Recent studies have reported the biological activities of the crude extracts/purified compounds from various parts of Garcinia cowa. In the present study, the dried fruit rinds of G. cowa were extracted with hexane and chloroform and the extracts were used to evaluate their antioxidant and antimutagenic activities. Using β-carotene-linoleate-model system, at 200 ppm concentration, hexane, chloroform extracts and butylated hydroxyanisole (BHA) showed 91.7, 93.7, and 98.0% antioxidant activity, respectively, whereas, at 50 ppm concentration the radical scavenging activity was 83.3, 86.3, and 88.5%, respectively, through DPPH method. At a concentration of 5000 μg/plate, hexane extract exhibited strong antimutagenicity against the mutagenicity of sodium azide in both the tester strains of Salmonella typhimurium (TA-100 and TA-1535). Chloroform extract showed strong antimutagenicity in both the tester strains at a concentration of 2500 μg/plate and above. However, the chloroform extract exhibited higher antioxidant and antimutagenic activities than that of hexane extract. This study showed that both the extracts from the fruit rinds of *G. cowa* possess antioxidant and antimutagenic properties.

2.11 Neuropharmacological Activity, Analgesic Activity

The current study was aimed to investigate the antibacterial as well as neuropharmacological and analgesic activities of methanol extract from the leaf and bark of *Garcinia cowa* (Family: Clusiaceae). The antibacterial activity test was performed by determining the zone of inhibition of living microorganisms compared with the standard drug, Ciprofloxacin. The result showed that, the petrolium ether, dichloromethane and methanol extracts from the leaf and bark have mild to moderate antibacterial activity. The neuropharmacological screening was evaluated by hole cross and open field tests where a significant and dose dependent suppression of motor activity and exploratory behavior was observed in the methanol extract of G. cowa when treated in Swiss Albino mice with the reference sedative drug, Diazepam. The analgesic activity was evaluated by using acetic acid-induced writhing test and tail immersion method at a dose of 200 and 400 mg/kg body weight. The results displayed 40.68% and 52.31% of inhibition for leaf and 56.18% and 56.91% for bark extract in the acetic acid-induced writhing test which is mild to the

reference standard drug, Diclofenac-Na whose writhing inhibition was 78.21%. The analgesic activity in the tail immersion method was dose dependent.

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

The prevalence of overweight and obesity has increased over the last decade, and current measures have not been able to stem the tide. A wide variety of weight management strategies are presently available, and some involve the use of dietary supplements marketed as slimming aids. One such slimming aid is *Garcinia* extract, (-)-hydroxycitric acid (HCA).

HCA is a derivative of citric acid and can be found in plant species native to South Asia such as *Garcinia cambogia*, *Garcinia indica*, and *Garcinia atroviridis* . HCA is usually marketed as a weight loss supplement either alone or in combination with other supplements. Some authors have suggested that HCA causes weight loss by competitively inhibiting the enzyme adenosine triphosphatase-citrate-lyas*(*Asia Pacific Journal of Clinical Nutrition. 2007;).

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

Two new xanthones, cowaxanthones G (**1**) and H (**2**), and 23 known analogues were isolated from an acetone extract of the leaves of *Garcinia cowa*. The isolated compounds were evaluated for cytotoxicity against three cancer cell lines and immortalized HL7702 normal liver cells, whereby compounds **1**, **5**, **8**, and **15**–**17** exhibited significant cytotoxicity. Cell cycle analysis using flow cytometry showed that **5** induced cell cycle arrest at the S phase in a dose-dependent manner, **1** and **16** at the G2/M phase, and **17** at the G1 phase, while **16** and **17** induced apoptosis. Moreover, autophagy analysis by GFP-LC3 puncta formation and western blotting suggested that **17** induced autophagy. Taken together, our results suggest that these xanthones possess anticancer activities targeting cell cycle, apoptosis, and autophagy signaling pathways(Xia ZX,2015).

2.13 Antibacterial, Neuropharmacological and Analgesic Activities of *Garcinia Cowa*

The current study was aimed to investigate the antibacterial as well as neuropharmacological and analgesic activities of methanol extract from the leaf and bark of *Garcinia cowa* (Family: Clusiaceae). The antibacterial activity test was performed by determining the zone of inhibition of living microorganisms compared with the standard drug, Ciprofloxacin. The result showed that, the petrolium ether, dichloromethane and methanol extracts from the leaf and bark have mild to moderate

antibacterial activity. The neuropharmacological screening was evaluated by hole cross and open field tests where a significant and dose dependent suppression of motor activity and exploratory behavior was observed in the methanol extract of *G. cowa* when treated in Swiss Albino mice with the reference sedative drug, Diazepam. The analgesic activity was evaluated by using acetic acid-induced writhing test and tail immersion method at a dose of 200 and 400 mg/kg body weight. The results displayed 40.68% and 52.31% of inhibition for leaf and 56.18% and 56.91% for bark extract in the acetic acid-induced writhing test which is mild to the reference standard drug, Diclofenac-Na whose writhing inhibition was 78.21%. The analgesic activity in the tail immersion method was dose dependent**(**Bloom F. E. 1996).

3.1Theory of Phytochemical Screening

3.1.1. 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.	

Table 3.1: List of reagent used for phytochemical screening

3.1.2 Test Compounds

DCM extract of bark of *Garcinia cowa*

3.1.3 Preparation of Sample Solution

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

3.1.4 Phytochemical Tests

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

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

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

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

iv. ***Borntragers's test for anthraquinone glycosides*:** 1 ml of sample solution was shaken with 5 ml of chloroform in a test tube for at least 5 minutes then again shaken with an

v. 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.

vi. ***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.

vii. ***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.

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

ix. ***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.

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

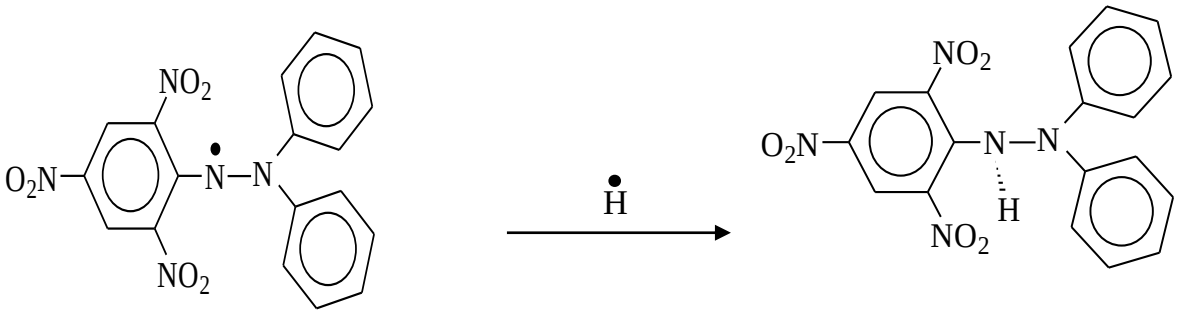
3.2 Assessment of In Vitro Pharmacological Property

3.2.1 Determination of Antioxidant property

3.2.1.1DPPH Free Radical Scavenging Assay (Braca *et al.,* 2001)

Principle

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



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

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

Table 3.2:List of reagent used for DPPH test

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

Preparation of Standard/ Extract solution

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

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

Table 3.3: Preparation of methanol extract of *G.cowa* stem or ascorbic acid solution

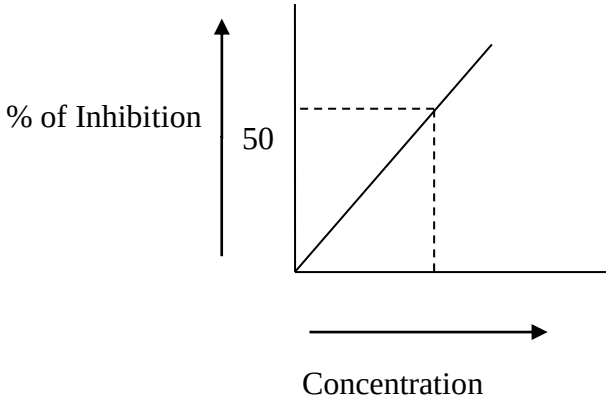
Procedure

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

Calculation

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

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.



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

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):

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

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

Table 3.4: List of reagent used for total phenol test

Preparation of 7.5% Sodium carbonate solution

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

Preparation of Standard solution

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

Concentration (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)	1000	2

Table 3.5: Preparation of Gallic Acid solution

Preparation of Extract solution

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

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

Table 3.6: Preparation of methanol extract of *Garcinia cowa* stem solution

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 calibrationcurve (mg/ml)

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

3.2.2 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).

3.2.2.1 Materials

3.2.2.1.1 Microorganisms

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

3.2.2.1.2 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:

3.2.2.1.3 List of Test Bacteria:

Bacillus sereus	*Vibrio parahemolyticus*
Bacillus megaterium	*Staphylococcus aureus*

Bacillus subtilis	*E.Coli*
Salmonella paratyphi	*Shigella dysenteriae*
Salmonella typhi	*Pseudomonas aureus*

Table 3.7: List of test bacteria

3.2.2.1.4 Culture Media and Chemicals

- Nutrient agar media
- Ethanol
- Chloroform

3.2.2.1.5 Equipments

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

- Incubator
- Refrigerator

3.2.2.1.6 Test Materials

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

3.2.2.2 Methods

3.2.2.2.1 Culture Preparation

Composition of culture media

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

Composition of Nutrient agar media (1000 ml)

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

To prepare required volume of this medium, calculated amount of each of the constituents was

taken in a conical flask and distilled water was added to it to make the required volume. The

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

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

3.2.2.2.3 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 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

3.2.2.2.4 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 petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media

3.2.2.2.5 Preparation of Discs

3.2.2.2.5.1 Standard discs

Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Amoxycillin (10µg/disc) standard disc was used as the positive control.

3.2.2.2.5.2 Blank discs

Blank discs were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves and did not influenced the results.

3.2.2.2.5.3 Preparation of sample discs with test samples

20 & 30 mg of each test samples were dissolved in 1 ml of methanol to obtain the concentration 20µg/µl&30µg/µl in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petridish under the laminar hood. Then discs were soaked with 10 µl of solutions of test samples containing 200 µg and 300µg of extract. Then the disks were dried.

3.2.2.2.6 Placement of Disc and Incubation

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

3.2.2.2.7 Determination of Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After

incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

4.1 Phytochemical Screening of DCM extract of *Garcinia cowa* Bark

Plant extract	Alkaloids	Terpenoids	Carbohydrats	Tannins	Flavonoids	Saponins	Glycosides
DCM extract	-	-	+	+	+	-	+

Table 4.1 : Result of Phytochemical Screening of DCM extract of *Garcinia cowa* Bark

4.2 DPPH Test of DCM extract of *Garcinia cowa* Bark

Concentration	Absorbance of sample	% of inhibition of sample
0	0	0
12.5	0.807	6.162791
25	0.599	30.34884
50	0.158	81.62791
100	0.14	83.72093
200	0.129	85

Concentration	Absorbance of ascorbic acid	% of inhibition of ascorbic acid
0	0	0
12.5	0.0986	70.66349301
25	0.0899	73.25200833
50	0.0647	80.74977685

100	0.0397	88.18803927
200	0.0245	92.710502

Table 4.2 : Result of absorbance and %of inhibition of DCM extract of *Garcinia cowa* bark and ascorbic acid .

4.2.1Preparation of DPPH Scavenging Activity Curve

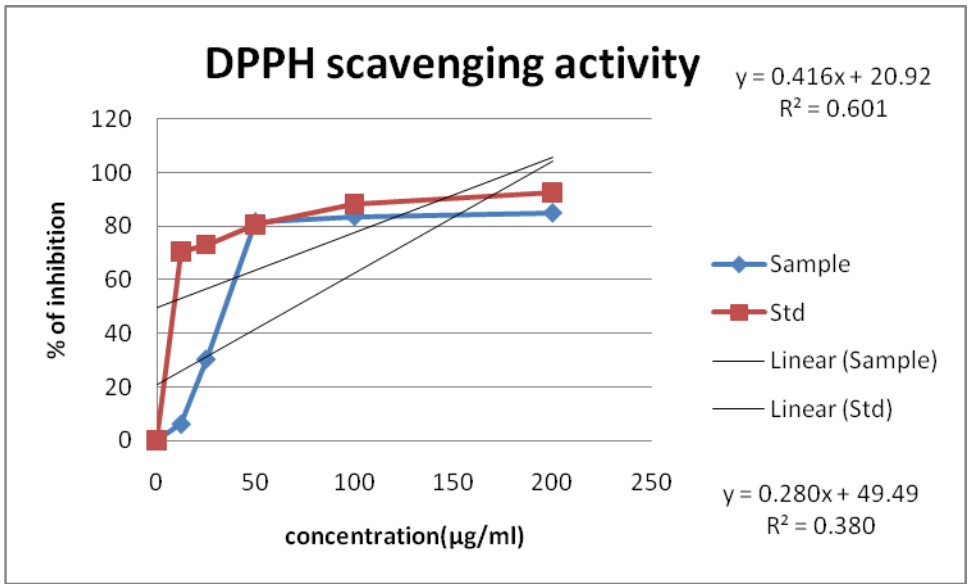


Figure 4.1: DPPH scavenging activity curve of DCM extract of *Garcinia cowa* bark.

4.2.2 Results of DPPH Test of DCM extract of *G.cowa* Bark

DCM extract of *G.cowa* Bark /Ascorbic acid	**Regression Line**	**R^2 Line**	**IC_{50} Value (µg/ml)**
DCM extract of *G.cowa* Bark	Y=0.416x+20.92	R^2=0.601	69.903
Ascorbic acid	Y=0.280x+49.49	R^2=0.380	1.821

Table 4.3 : Result of DPPH test of DCM extract of *Garcinia cowa* bark

4.3 Total Phenol content of DCM extract of *Garcinia cowa*

4.3.1 Preparation of Standard Curve for Gallic Acid:

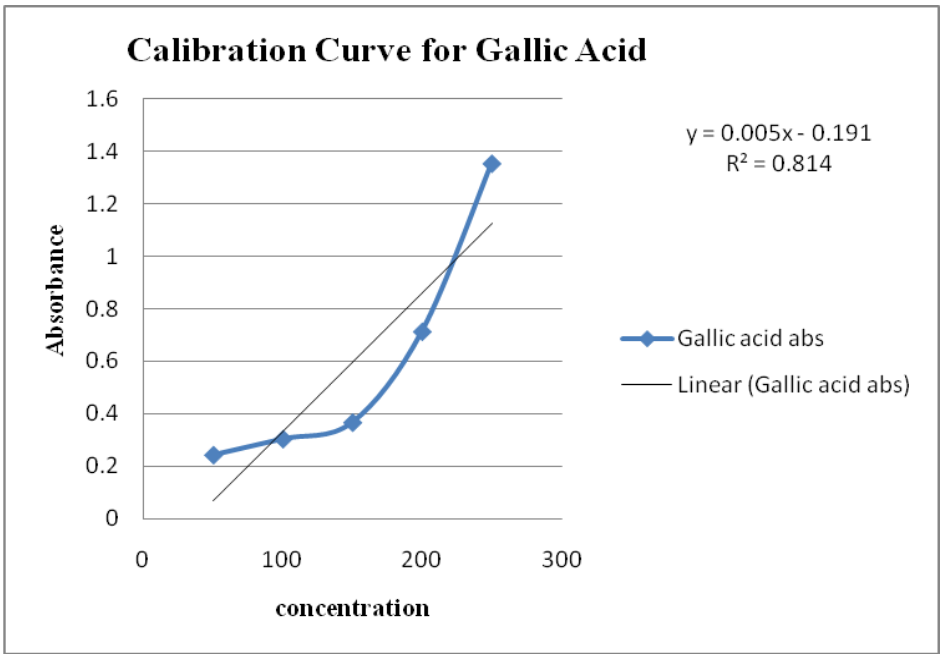


Figure 4.2 : Standard Curve of Gallic Acid

4.3.2 Results of Total Phenol content

Bark DCM Absorbance	Y=0.005x-0.191	Mean	Standard Deviation
1.582	354.6		
1.728	383.8	362.9333	18.19267
1.561	350.4		

Table 4.4: Result of Total Phenol content of DCM extract of *Garcinia cowa* bark

The total Phenol content was 362.9333±18.19267 mg/g equivalent to Gallic Acid for DCM extract of *Garcinia cowa* Bark

4.4 Antimicrobial screening of DCM extract of *Garcinia cowa* Bark

Name of microorganism	**Zone of inhibition**		
	DCM extract of G.cowa bark (400µg/disc)	**DCM extract of G.cowa bark (800µg/disc)**	**Kanamycin (30µg/disc)**
Bacillus sereus	7	8	30
Bacillus megaterium	8	8	25
Bacillus subtilis	-	9	28
Salmonella paratyphi	-	9	40
Salmonella typhi	7	8	26
Vibrio parahemolyticus	7	-	26
Staphylococcus aureus	-	-	25
E.Coli	-	-	35
Shigella dysenteriae	-	-	25
Pseudomonas aureus	-	-	30

Table 4.5 : Result of zone of inhibition of DCM extract of *G. cowa bark* and Kanamycin

Discussion

The preliminary phytochemical analysis indicated the presence of the group of compounds such as alkaloids,,saponins,tanins,terpenoids,carbohydrates,flavonoids,tanin and steroids.Many compounds belonging to these secondary metabolite groups have been reported to their antimicrobial activities.

The antioxidant activity was measured by DPPH scavenging assay and total Phenol content. The IC_{50} values of DPPH scavenging assay was 69.903 µg/ml for DCM extract of *G.cowa* bark. The Total Phenol content was 362.93±18.19mg/g equivalent to Gallic Acid for DCM extract of *Garcinia cowa* bark.The result are express as mean ± standard deviation where the n=3. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity.

The Antimicrobial Activity of the DCM extract of *Garcinia cowa* was tested against ten microorganism. The highest antimicrobial activity was shown against *Vibrio parahemolyticus* and*salmonella typhi,Bacillus sereus.* The diameter of zone of inhibition was 7 mm(400µg/disc).It showed moderate activity against *Bacillus subtilis(9mm) and Bacillus megaterium(8mm).*In case of 800µg/disc, the highest zone of inhibition of *Garcinia cowa* was 9mm for *Salmonella typhi* where the standard Kanamycin zone of inhibition was 26 mm. It also showed good activity against *Bacillus megaterium* (8mm) *and Pseudomonas aureus* (9mm).It showed no activity against *E coli, Salmonella paratyphi* and *Staphylococcus aureus,shigella dysenteriae,pseudomonas aureus.* So, the DCM extract of the *Garcinia cowa* showed good antimicrobial activity against the selected microorganisms. Thus, further extensive investigations are necessary to find out the active principles present in these plants.

Conclusion

Significant aspect of the study is the finding of plant of *Garcinia cowa* based highly active of an antimicrobial compounds, active against several pathogenic microbes.*Garciniacowa* extract had the broadest antimicrobial activity. It inhibited growth of 8 bacterial species.In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect.the results indicate that *Garcinia cowa* of stem bark could be one of important sources of natural cytotoxic compounds. The potential value of the stem bark of *G. cowa* from the West Sumatra region of Indonesia as a source of cytotoxic.compounds has been reported for the first time here. Five known and one new xanthones were isolated from thesteam bark of *Garcinia Cowa*.The plants of this genus are also a rich source of xanthones, many of which exhibit interesting biological and pharmacological activities such as antibacterial and antioxidant properties.

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