

Determination of total flavonoid content of different parts of *Garcinia cowa*

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 CANDIDATE

Samonty Biswas hereby declare that this dissertation, entitled **Determination of total flavonoid content of different parts of *Garcinia cowa*** 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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This is to certify that the dissertation, entitled '**Determination of total flavonoid content of different parts of *Garcinia cowa***' is a research work carried out by Samonty Biswas (ID: 2013-3-70-009) 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 '**Determination of total flavonoid content of different parts of *Garcinia cowa***' by Samonty Biswas (ID: 2013-3-70-009), 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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ACKNOWLEDGEMENTS

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

I am thankful to my honorable teacher and supervisor, **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, for his amiability to provide me with untiring guidance, whole cooperation and for his extensive knowledge in research that helped me in all the spheres to perform the research work.

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

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

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

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

Thank you.

Dedication

I dedicate this research to my beloved Grandmother,my Father,my Uncle Dr Arun Kumar and teachers, who taught me to think, understand and express. I earnestly feel that without their inspiration,able guidance and dedication, I would not be able to pass through the tiring process of this research.

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ABSTRACT

Garcinia cowa have been used in traditional medicine in several parts of the world for treatment of the most different illnesses. In Thailand, *G. cowa* have been widely used in Thai folk medicine. saponins, tanins, terpenoids, carbohydrates, flavonoids groups are present. Flavonoid are ubiquitous in photosynthesizing cells and are commonly found in fruit, bark, leaf nuts, seeds, stems. Increasingly, Natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity. Further investigations needed to identify the active constituents and the exact mechanism of action responsible for reported antioxidant and antimicrobial properties of *Garcinia cowa*. *Garcinia cowa* is a medicinal plant enriched with various chemical constituents having different medicinal activities. The different parts of *Garcinia cowa* as stem, leaves, bark are rinse in DCM and Methanol than get extract for Flavonoid test. The total Flavonoid content of methanol leaf was 14.91 ± 0.06 mg/g QE. The result are express as mean \pm standard deviation. The total Flavonoid content of methanol stem was 20.22 ± 0.036 mg/g QE. The total Flavonoid content of Pet Leaf was 77.11 ± 0.10 mg/g Quercetin(QE). The total Flavonoid content of pet stem was 79.88 ± 0.061 mg/g QE. The total Flavonoid content of DCM Leaf was 83.77 ± 0.009 mg/g QE. The total Flavonoid content of DCM Stem was 31.55 ± 0.032 mg/g QE. The result is much good than previous experiments but further extensive investigations are necessary.

Key words: *Garcinia cowa*, Antimicrobial activities, Antioxidant activities, DCM extract of *Garcinia cowa*, Methanol extract of *Garcinia cowa*, Total Flavonoid conten

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 Vaidis 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,

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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.

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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 *honour* 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. Apart 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.

1.2 Medicinal Plant:

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.

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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 (Greene, Marjorie, 2004).

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 (*Coffea arabica*) 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

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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 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 Dioscorus of Carystus, written during the 1st century B.C, and one by Crateus 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).



MEDICINE IN ANCIENT EGYPT—reproduced here is one of a series of original oil paintings, "A History of Medicine in Pictures," commissioned by Parke-Davis.

Great Moments in Medicine

Clothed in spotless linens and wearing a wig, as became the dignity of his status, an Egyptian physician of 1500 B.C. administers to a patient with symptoms of lockjaw. Though Egyptian doctors dominated medicine in the ancient world for thousands of years, this highly-respected practitioner could rely only on personal skill, judgment, and experience to combat such dreaded killers as tetanus.

Today, 3500 years later, due to advances in pharmaceutical research,

tetanus is no longer a source of fear. The modern physician employs safe, effective immunizing agents to protect you and your family from tetanus, polio, and many other infections that were killers of defenseless persons in former times.

Parke-Davis scientists are proud of their place in the living history of modern medicine, helping to provide the people of the world with the better health and longer life that come with better medicines.

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...Pioneers in better medicines

Figure 1.1 : Great Moments in Medicine

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people (Balick and Cox, 1996). Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Fabricant and Farnsworth, 2001).



Figure 1.2 : Medicine in Ancient Times

1.5 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.

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- 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, sheetroj 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.

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- 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: Euphorbiaceae	Fruit	Vitamin C, cough, Diabetes, cold, Laxative, hyperacidity
	Ashok Family: Caesalpiniaceae	Bark, flower	Menstrual, Pain, Uterine, Diabetes
	Aswagandha Family: Solanaceae	Root, leaf	Restorative tonic, Stress, Nerves disorder, Aphrodisiac
	Bael Family: Rutaceae	Fruit, Bark	Diarrhoea, Dysentery

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	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

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	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,Snake 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

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	Rakta chitrak Family: Plumbaginaceae	Root	Inflammation, Cough
	Kochila Family: Loganiaceae	Seed	Nervous, Paralysis, Wound
	Harida Family: Combretaceae	powder	Ulcer, Leprosy, Cough
	Bahada	Seed, Bark, Fruit	Insomnia, Vomiting
	Neem Family: Mahaceae	Rhizome	Urinary, Sedative, Hypertensive
	Bach Family: Araceae	Rhizome	Sedative, Analgesic, Hypertensive
	Nageswar champa Family: Guttiferae	Bark, Leaf, Flower	Asthma, Skin burning, Vomiting, Piles
	Kurai Family: Apocyanaceae	Bark seed	Anti-pyretic, Dysentery
	Dalchini Family: Lauraceae	Bark oil	Bronchitis, Asthma, Cardiac disorder, Fever

Table 1.1: List of important medicinal plants and their usages

1.6 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), a swiss 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 (*Erythroxyllum* 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).

1.7 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).

1.8 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.

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2. Traditional Chinese medicine has been chosen by the World Health Organization for worldwide propagation to meet the health 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).

1.9 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

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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

1.9.1 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 feverfew etc.
- B. Culinary Herbs are probably the mostly used as cooking herbs because of their strong flavours like 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.

1.10 Global Scenario of Medicinal Plants

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal medicine is a common element in Ayurvedic, homeopathic, naturopathic, traditional and oriental, Native American & Indian medicine. Plant products also play an important role in the health care

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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 15 percent 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).

1.11 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, inflammatory 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).

1.11.1 Classification of Antioxidants

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

1.11.2 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.

1.11.3 Enzymatic Antioxidants

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

1.11.4 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_2^-) 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).

1.11.5 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.

1.11.6 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, carotenoids, polyphenols, and other antioxidants as listed below.

1.11.6.1 Carotenoid

Carotenoid consists of β-carotene, lycopene, lutein, and zeaxanthin. They are fat-soluble colored compounds found in fruits and vegetables. β-Carotene is found mostly in redish-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).

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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.

1.11.6.2 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 peroxy 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).

1.11.6.3 Flavonoid

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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.

1.11.6.4 Minerals:

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

1.11.7 Iron (Fe):

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

1.11.8 Magnesium (Mg):

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

1.11.9 Selenium (Se):

Selenium is also a very important component of enzymatic antioxidant. In the presence of selenium (Se), glutathione peroxidase (GPx) plays a protective role against oxidation of lipid and protects the cell membrane and takes part in H₂O₂ and lipids' hydroxyperoxide metabolism. Hence, Se behaves like vitamin E and can be substituted in place of vitamin E and is used to prevent the risk of cancer and cardiovascular disease *Copper (Cu), Zinc (Zn), and Manganese (Mn)* SOD is a class of enzyme that consists of different types of SODs, depending upon their

metal cofactor such as Cu–Zn and Mn. Cu–Zn SOD is found in the cytosol having Cu and Zn at their active sites which helps in proton conduction, whereas Mn-SOD is found in mitochondria and has Mn at its active site. These metals are responsible for SOD's antioxidant activities.

1.11.10 Vitamins

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

1.11.11 Vitamin A:

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

1.11.12 Vitamin C :

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

1.11.13 Vitamin E:

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

1.11.14 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.

1.11.15 Bilirubin:

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

1.11.16 Uric Acid:

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

1.11.17 Coenzyme Q:

Coenzyme Q is also known as ubiquinol (Co Q) and is an oil-soluble antioxidant. This is produced in the body through monovalent pathway, in heart, liver, kidney, pancreas, etc.

1.12 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

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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*

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

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Figure 1.3 : Habitat of Garcinia Class Plants Around the World



Figure 1.4: *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

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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, is the floral emblem of Mobuto and Tarama on Okinawa. The Malaysian town of Bruas – 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, dull 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).

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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).



Figure 1.5: 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

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thesetwo 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 (1,2-dihydroxypropane-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*

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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 Classes of Compounds Isolated from *G.cowa*:

1.15.1 Flavonoids: Twelve flavonoids were isolated from *G. cowa* with garccowasides A (6), B (7) and C (8) being first reported as new compounds. Of these compounds, only morelloflavone and morelloflavone-7-O-glucoside showed strong antioxidant activities. Phloroglucinols

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Phloroglucinols are based on a phloroglucinol or 1,3,5-benzenetriol core skeleton or its 1,3,5-cyclohexanetrione (phloroglucin) tautomer. The phloroglucinols found in *G. cowa* have a benzoyl group and geranyl and polyprenyl units as substituent groups. So far, fifteen phloroglucinols have been obtained from the twig including six new compounds: guttiferone K (15a), chamuangone (16), garcicowins A (17), B (18), C (21) and D (22), and nine known phloroglucinols: cambogin (14), guttiferones K (15b), B (25) and F(26), oblongifolins B (19), C (20), A (24) and D (27), and 30-epicambogin (23). Some of them showed selective cytotoxicity against two cancer cell lines (HT-29 and HCT-116) and normal colon cells (CCD-18Co). Guttiferone K (15) and 30-epicambogin (23) exhibited highest cytotoxicity against cancer cell line HT-29 [7]. The name guttiferone K has been given to two different structures in the literature [7, 26] as shown in Table 1. Only one compound, chamuangone (16), was tested for its antibacterial activity and was found to be active against *S. pyogenes* (MIC = 7.8 µg/mL), *S. viridans* and *H. pylori* (MICs = 15.6 µg/mL), and *S. aureus*, *B. subtilis* and *Enterococcus* sp. (all of this bacteria shown MICs = 31.2 µg/mL) [27]. Terpenes and Steroids Terpenes and steroids represent two large classes of natural products, although they are rare in *G. cowa*. Only four of these types of compounds (5% of the total compounds isolated) were present in *G. cowa*, viz. friedelin, daucosterol, -sitosterol and stigmasterol. None of these compounds were further studied for their biological activities. However, these compounds which which were isolated from other plants had been investigated for their biological activities. Friedelin from the root bark of *Terminalia avicennioides* exhibited antibacterial activity against *Bacillus Calmette-Guerin* (BCG) with an MIC of 4.9 µg/mL. Friedelin and stigmasterol isolated from the leaf of *Jatropha tanjorensis* were tested against human pathogenic microorganisms, i.e. Gram-positive bacteria: *Bacillus cereus*, *B. subtilis*, *S. aureus* and *S. epidermis*; Gram-negative bacteria: *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *P. vulgaris*, *Salmonella paratyphi*, *S. paratyphi A*, *Vibrio alcaligenes* and *V. cholera*; and fungi: *Aspergillus fumigatus*, *Candida albicans*, *Microsporium gypseum* and *Trichophyton rubrum* using the agar-well diffusion and disk diffusion methods. Friedelin, at the concentration of 2 µg/mL, showed maximum activity with 37-40, 17-40 and 31-33 mm of clear zone diameter against these three types of microorganisms respectively, while stigmasterol at the same concentration exhibited maximum activity with 13-15, 8-17 and 7-8 mm of clear zone

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

1.15.2 Antibacterial activity:

Eight xanthenes from the fruit: cowaxanthenes B (34) and C (39), 7-O-methylgarcinone E (36), -mangostin, \square -mangostin, mangostanin, cowanol and cowanin were investigated for their antibacterial activity against *S. aureus* and MRSA. \square -Mangostin and mangostanin showed significant activity against these bacteria. \square -Mangostin had a MIC value of 8 $\mu\text{g/mL}$ against both *S. aureus* and MRSA while mangostanin had an MIC value of 4 $\mu\text{g/mL}$ against both bacteria.

1.15.3 Anti-inflammatory activity:

Eight xanthenes: cowaxanthenes A (32), B (34), C (39) and D (42), \square -mangostin, mangostanin, cowanol and cowanin were tested for their anti-inflammatory activity using the ethyl phenylpropiolate induced ear edema assay. All xanthenes except cowanol were more active than the standard drug, phenylbutazone. Antimalarial activity Five xanthenes isolated from the stem bark: 7-O-methylgarcinone, \square -mangostin, cowaxanthone, cowanol and cowanin had significant in vitro antimalarial activity against *Plasmodium falciparum* with IC₅₀ values ranging between 1.5-3.0 $\mu\text{g/mL}$. Anticancer activity Six xanthenes: cowaxanthone, cowanol, cowanin, norcowanin, 3,6-di-O-methyl- \square -mangostin and dulxanthone isolated from twig were evaluated for their cytotoxicity against NCI-H187, KB, MFC-7 and/or HepG2 cell lines. Cowaxanthone, cowanin, norcowanin and 3,6-di-O-methyl- \square -mangostin exhibited significant cytotoxicity against the NCI-H187 cell line with IC₅₀ values ranging between 3.87-8.58 $\mu\text{g/mL}$, and moderately inhibited KB and MCF-7 cancer cell lines with IC₅₀ values ranging between 6.43-15.43 and 10.59- 21.38 $\mu\text{g/mL}$ respectively. Dulxanthone was found to be cytotoxic against the HepG2 cell line.

1.16 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 decreases 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.

1.17 Resource of natural products for establishment of new drugs

Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Nature has been a source of therapeutic agents and a significant number of modern drugs have been developed from natural sources, many based on their use in traditional

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medicine. Over the last century, a remarkable number of top selling drugs have been derived from natural products (Vincristine from *Catharanthus roseus*, morphine from *Papaver somniferum*, quinine and quinidine from *Cinchona spp.*) Nowadays, approximately 40% of the modern drugs have been developed from natural source (Sarker et al. 2006). More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anti –cancer drugs were from natural origin. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancer had natural origin. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporine and paclitaxel) 1) Of the 30 top - selling medicines were natural products or their derivatives (Newman et al. 2007). In light of all these facts, natural product drug discovery process failed to generate little respect. As drug discovery has emerged into a highly competitive era in which the quality of chemical collections and the time taken from assay to drug development are crucial factors in the success of a company, combinatorial chemistry has become the darling of the pharmaceutical industry, bringing with it the promise of new level of chemical diversity (Strohl 2000). But this adoption of new strategy by the pharmaceutical companies gained little momentum. Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering and met genomic approaches to identify novel natural product lead molecules have met with limited success. These disappointments have led the pharmaceutical industry to consider whether natural product chemical diversity can or will continue to generate valuable templates for drug development

Natural products offer a potentially infinite source of chemical diversity unparalleled to any synthetic chemical collection or combinatorial chemistry approach. In addition to that, these potent natural product compounds can have astounding chemical structures that can lead to unexpected, alternative medicinal chemistry programs based on important biological targets (Strohl 2000). In the past few years, new natural products with a wide variety of chemical classes have been reported in the scientific literatures. Moreover, a total of 19 natural product based drugs were approved for marketing worldwide in between the year 2005 to April 2010, among which 7 being classified as natural products, 10 semi-synthetic natural products and 2 natural product derived drugs

1.18 Approaches of drug development

The major portion of the present day knowledge of the medicinal properties of plants is the sum total of some observations and experiences. According to some generous estimates, almost 80 percent of the present day medicines are directly or indirectly obtained from plants (Ghani 1998).

Steps of drug development from plant sources given below: Selection of plant species:

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

Evaluation of toxicity:

- ❖ Gather data concerning toxicity and if demonstrate no toxicity then proceed to next step
- ❖ If toxicity data is not exit, select an appropriate test for toxicity analysis
- ❖ Develop and prepare bioassay protocol for safety and toxicity

Preparation of plant sample and element analysis:

- ❖ Collection of plant sample
- ❖ Extraction
 1. Compare the selective and yield
 2. Use various extraction technique
- ❖ Analysis for elemental contents

Biological Testing:

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

Isolating active compounds:

- ❖ Isolating and characterization of compounds responsible for
- ❖ Observed biological activity

- ❖ Evaluation of active compounds singularly and in combination with others to explore existence of activity and/or synergy of biological effect

In-vivo analysis:

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

Commercialization:

- ❖ Develop appropriate dose delivery system.
- ❖ Analyze cost-effectiveness
- ❖ Sustainable industrial production

1.19 Aim of this experiment

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs ,problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity. The main objective of this study was to discovery of new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Garcinia cowa is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for

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conducting the study, this research work was performed on this plant. The principal aim of the present study was to investigate the scientific basis of the traditional uses of the plant. The methanolic extract of to evaluate their in- vitro pharmacological activities (like antioxidant, antimicrobial).

Study Area:

The research was carried out in the Research Lab, Microbiology Lab and Pharmacognosy Lab of Department of Pharmacy, East West University, Dhaka

Data Collection:

All the relevant data has been collected from two types of sources:

1. Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.
2. Secondary sources: various publications like journals, papers, documents and websites

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Literature Review on *Garcinia cowa*:

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

2.1 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).

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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 antitumor promoting (Mukarami *et al.*, 1995), inhibition of human low density lipoprotein peroxidation and anti-platelet activities have been reported on the crude extract of leaves. 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*-xanthone (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 xanthenes respectively. Seventy eight of these compounds have been identified from the plant and several have interesting pharmacological activities (Laphookhieo, S. Pyne, S. G 2013).

2.3 Antimalarial Xanthenes from *Garcinia Cowa*

Five xanthenes 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 μ M) 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 μ M. 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 μ M. 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 μ g/g) in *Garcinia xanthochymus* and total antioxidant activity in terms of IC₅₀ value was found highest (11.61) in

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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/in² 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 xanthenes 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* (IC₅₀s 250-500 µg growth of the Gram-positive bacteria (IC₅₀s 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 was superoxidation induced by copper ions. The twig extract had an IC₅₀ value of 20.5 g/mL than the leaf extract (IC₅₀ not measured). The more 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 absorption 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 IC₅₀ 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

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extracts. At the concentration of 100 µg/ml, the hexane extract and dichloromethane extract inhibited cholesterol absorption 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 IC₅₀ 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:

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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 petroleum 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-lyase (Asia Pacific Journal of Clinical Nutrition. 2007;).

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

Two new xanthenes, cowaxanthenes 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

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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 xanthenes 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 petroleum 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).

2.14 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

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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.

Material & Methods

3.1 Collection & Preparation of Plant Material

Garcinia cowa plant was collected in the month of June, 2014 from Hill tracts during rainy season when weeds were in their maximum densities. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried leaves were then ground into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Washing and Drying of *Garcinia cowa*

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc within them. The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven **Determination of Total Flavonoids Content** (Wang and Jiao, 2000) 50°C for 2 hours.

3.3 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 700 gm which was measured using electronic balance and it was found to be 700 gm.

3.4 Extraction of the Dried Powdered Sample

The fine powder of *Garcinia cowa* leaves was dissolved in 7L methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass

jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar

Material & Methods

Evaluation of sedative & hypnotic activities

hole board method

3.5 Filtration of the Extract

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

3.6 Evaporation and Condensation of the leaf extracts

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

3.7 Principle of a Rotary Evaporator

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

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EYELA. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L-50L) versions are used in pilot plants in commercial chemical operations.



Fig 3.1: Rotary Evaporator

3.8 Principle

Total flavonoid was determined using the Aluminum chloride colorimetric method described by Wang and Jiao (2000). The principle of aluminum chloride colorimetric method is that, aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. In preliminary experiments, the wavelength scans of the complexes of 15 standards with aluminum chloride showed that the complexes formed by flavonols with C-3 and C-5 hydroxyl groups, such as galangin, morin and kaempferol, as well as those with extra ortho-dihydroxyl groups, such as rutin, quercetin, quercitrin and myricetin, had maximum absorbance at 415-440 nm (Chang *et al.*, 2002). However, the λ_{\max} of the complexes formed

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by chrysin and apigenin which have only the C-5 hydroxyl and C-4 keto groups were at 395 and 385 nm, respectively. Another flavone compound investigated, luteolin, which has the C-5 hydroxyl group and the ortho-dihydroxyl groups in B ring formed a complex that showed a strong absorption at 415 nm. In compromise, therefore, the wavelength 415 nm is chosen for absorbance measurement.

Reagent	Source
Aluminium Chloride	Fine Chemicals, India E.
Potassium Acetate	Merck (India) limited
Methanol	Merck, Germany
Quercetin	Sigma Chemicals, USA

3.9 Preparation of 10% Aluminium chloride (AlCl₃) solution

10 gm of AlCl₃ was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.10 Preparation of 1M Potassium acetate solution

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

3.11 Preparation of Standard solution

Quercetin stock solution of concentration 5µg/µl was prepared by dissolving 0.025 gm of quercetin into 5 ml of ethanol. The experimental concentrations from the stock solution were prepared by the following manner:

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Concentration ($\mu\text{g/ml}$)	Solution taken from stock solution (μl)	Solution taken from others	Adjust the volume by distilled methanol (μl)	Final volume (ml)
100	40	-	1960	2
50	-	1 ml (100 $\mu\text{g/ml}$)	1000	2
25	-	1 ml (50 $\mu\text{g/ml}$)	1000	2
12.5	-	1 ml (25 $\mu\text{g/ml}$)	1000	2
6.25		1 ml (12.5 $\mu\text{g/ml}$)	1000	2

3.12 Preparation of Extract solution

0.025gm of each plant extracts were dissolved into 5 ml of Ethanol to make the concentration of each solution 5 $\mu\text{g}/\mu\text{l}$ of plant extract. These solutions were considered as stock solutions. The experimental concentration from these stock solutions was prepared by the following manner:

Concentration ($\mu\text{g/ml}$)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled water (μl)	Final volume
200	40 μl	-	960	1.0 ml

3.13 Experimental Procedure

1. 1.0 ml of plant extract (200 $\mu\text{g/ml}$) or standard of different concentration solution was taken in a test tube.

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2. 3 ml of methanol was added to the test tube.
3. Then 200 μ l of 10% aluminium chloride solution was added into the same test tube.
4. Followed by the addition of 200 μ l of 1M potassium acetate solution into the test tube.
5. Finally, 5.6 ml of distilled water was mixed with the reaction mixture.
6. The reaction mixture then incubated for 30 minutes at room temperature to complete the reaction.
7. Then the absorbance of the solution was measured at 415 nm using a spectra photometer against blank.
8. Methanol served as blank.
9. The Total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following formula equation

$C = (c \times V)/m$, where;

C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent

c = the concentration of quercetin established from the calibration curve in mg/ml

V = the volume of extract in ml

m = the weight of crude plant extract in gm

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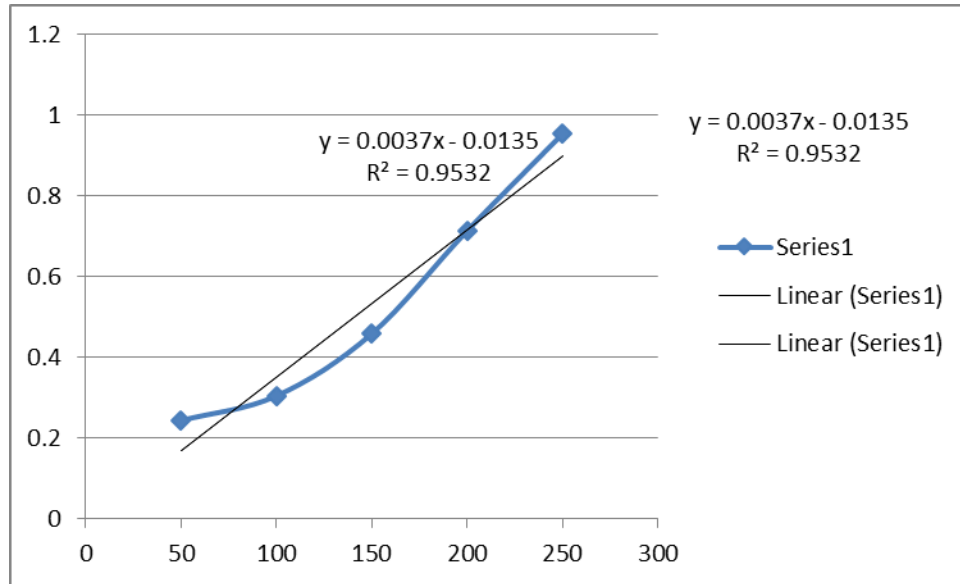


Fig:Calibration curve for quercetine

Table 4.1:Total Fiavonoid content of DCM Bark extract of *Garcinia cowa*

DCM Absorbance	Bark	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.224		79			
0.220		77.66667	78.77	0.003055	78.77 ± 0.003
0.226		79.66667			

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Table4.2: Total Flavonoid content of DCM Leaf extract of *Garcinia cowa*

DCM Leaf Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.249	87.33333			
0.234	82.33333	83.7777	0.00929	83.77 ± 0.009
0.232	81.6666			

Table4.3: Total Flavonoid content of Pet stem extract of *Garcinia cowa*

Pet Stem Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.232	81.66667			
0.220	77.66667	79.88889	0.0611	79.88 ± 0.061
0.228	80.33333			

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Table 4.4: Total Flavonoid content of Pet Leaf extract of *Garcinia cowa*

Pet Leaf Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.230	81			
0.215	76	77.11111	0.1040	77.11 ± 0.10
0.210	74.3333			

4.5: Total Flavonoid content of Methanol Stem extract of *Garcinia cowa*

Methanol Stem Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.018	10.3333			
0.036	16.3333	20.2222	0.03690	20.22 ± 0.03
0.089	34			

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Table4.6: Total Flavonoid content of Methanol stem extract of *Garcinia cowa*

Methanol Leaf Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.041	18			
0.054	22.33333	14.91667	0.06658	14.91 ± 0.06
0.045	19.33333			

Table4.7: Total Flavonoid content of Methanol Leaf extract of *Garcinia cowa*

DCM Stem Absorbance	X	Mean	Standard Deviation	Total flavonoid content mg/g QE
0.084	32.33333			
0.078	30.33333	31.55555	0.0032	31.55 ± 0.003
0.083	32			

Determination of total flavonoid content of different parts of *Garcinia cowa*

The preliminary phytochemical analysis indicated the presence of the group of compounds such as alkaloids, saponins, tanins, terpenoids, carbohydrates, flavonoids, and steroids. Herbal medicines have received high interest as a substitute to clinical treatment, and the demand for herbal remedies has currently increased rapidly. The increase in the number of herbal users as opposed to the insufficiency of scientific evidences on its safety has raised concerns regarding its detrimental effects and related concerns apply to the *Garcinia cowa* in this study. *Garcinia cowa* is a medicinal plant enriched with various chemical constituents having different medicinal activities. Flavonoids are ubiquitous in photosynthesizing cells and are commonly found in fruit, bark, leaf, nuts, seeds, stems, and flowers. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Further investigations are needed to identify the active constituents and the exact mechanism of action responsible for reported antioxidant and antimicrobial properties of *Garcinia cowa*. *Garcinia cowa* is a medicinal plant enriched with various chemical constituents having different medicinal activities. The different parts of *Garcinia cowa* as stem, leaves, bark are extracted in DCM and Methanol for Flavonoid test. The total Flavonoid content of methanol leaf was 14.97 ± 0.068 mg/g. The results are expressed as mean \pm standard deviation. The Flavonoid content of methanol stem was 20.22 ± 0.036 mg/g. The total Flavonoid content of Pet Leaf was 77.11 ± 0.14 mg/g. The total Flavonoid content of pet stem was 79.89 ± 0.061 mg/g. The total Flavonoid content of DCM Leaf was 83.77 ± 0.092 mg/g. The total Flavonoid content of DCM Stem was 31.55 ± 0.032 mg/g. Total Flavonoid content of different parts of *Garcinia cowa* was not determined previously, more investigation is needed because it scavenges the activity. Thus, further extensive investigations are necessary to find out the active principles present in these plants because it is used as a medicine for the treatment of various diseases. Dried fruits are shipped from Singapore to Calcutta and to China for medicinal use. The sliced and dried rind is powdered and administered to overcome dysentery. Made into an ointment, it is applied on eczema and other skin disorders.

6. 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.. The potential value *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 xanthenes were isolated from the stem bark of *Garcinia Cowa*. The plants of this genus are also a rich source of xanthenes, many of which exhibit interesting biological and pharmacological activities such as antibacterial and antioxidant properties. For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. In my experiment different parts *Garcinia Cowa* shows very positive result in flavonoid test. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

7. References

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