Antioxidant and Antimicrobial Investigations of Methanol Extract of *Garcinia cowa* Stem

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

Submitted By:

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DECLARATION BY THERESEARCH CANDIDADE

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

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled '**Antioxidant and Antimicrobial Investigations of Methanol Extract of *Garcinia cowa* stem**' is a research work carried out by Nishat Zahan (ID: 2013-1-70-001) in 2017, under the supervisionand guidance of me, in partial fulfillment of the requirement for the degree ofBachelor of Pharmacy. The thesis has not formed the basis for the award of anyother degree/diploma/fellowship or other similar title to any candidate of anyuniversity.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled is a research work carried out '**Antioxidant and Antimicrobial Investigations of Methanol Extract of *Garcinia cowa* stem**' by **Nishat Zahan** (ID: 2013-1-70-001), under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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Dedication

This Research Paper is dedicated to my beloved parents and my family members, they are my biggest inspiration.

Abstract

Garcinia cowa have been used in traditional folk medicine in Malaysia, Thailand and Myanmar. It is an abundant source of bioactive phytochemicals. Phytochemical investigations of the plant parts indicated that the fruit, twig and stem are the best source of secondary metabolites, providing flavonoids, phloroglucinols and xanthones respectively. *Garcinia cowa* has been used as a medicinal plant for the treatment of various diseases like dysentery, Stomach ache, Cramps, Headache. It is effective as an astringent, used in spasm. Many reports are available on the antiviral, antibacterial, antifungal, and anti-inflammatory, antimalarial properties of plants. The aim of the present study was to evaluate the antioxidant and antimicrobial activity of methanol extract of *Garcinia cowa* stem. The antioxidant activity was measured by DPPH scavenging assay and total Phenol content test. The IC_{50} values of DPPH test was 52.358µg/ml for methanol extract of *G. cowa* stem. The Total Phenol content was 149.6±10.57mg/g equivalent to Gallic Acid for methanol extract of *Garcinia cowa* stem. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. The antimicrobial activities of methanol extract of *Garcinia cowa* stem were tested against ten microorganisms by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The methanol extract of *Garcinia cowa* stem showed good (7mm-11mm) antimicrobial activities against themicroorganisms. Methanol extract of *Garcinia cowa* stem showed highest activity against *Salmonella typhi* and moderate activity against *Bacillus megaterium, Pseudomonas aureus, Bacillus subtilis,* and *E.Coli.* No activity was found against *Bacillus sereus, Salmonella paratyphi* and *Staphylococcus aureus.* In conclusion, further investigations are needed to identify the active constituents and the exact mechanisms of action responsible for the reported antioxidant and antimicrobial properties of *Garcinia cowa* stem.

Key Words: Medicinal Plant, *Garcinia cowa*, Secondary metabolites, Antioxidant, DPPH, Total Phenol, Antimicrobial.

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Chapter-1

Introduction

1.1 Human and Plant

The relationship between human and plants has always been profoundly important. Plants affect every aspect of our lives and without them life would not be possible at all. Plants not only regulate the concentration of gases in the air (making it 'breathable'), but also capable of transforming sunlight into food energy, which all other forms of life ultimately depend upon. Some have termed them 'original alchemists', (or less poetically -biochemical factories) capable of producing a myriad of chemical compounds, which modern chemists still find difficult to imitate. Since the stone-age human beings of all cultures and races have been incredibly innovative with regard to utilizing plant materials for their various needs. Plants provide a sheer in exhaustible source of widely varying materials-timber for building shelters and making tools, fibers for twine and rope, for weaving and basket making, paper, natural dyes, perfumes, resins, oils and soap. Plants, of course, also offer wide varieties of food for both body and soul, as well as multitudes of medicines for all types of diseases, from the common cold to cancer. Phytochemical compounds have played a significant role in the development of many important drugs that have saved thousands of lives over the centuries, and the hunt for new plant medicines is still on. In fact, presently it is a race against time. As more and more habitats of rich biodiversity are threatened by the forces of development, scientists all over the world are scrambling to identify new plant species and to learn about their traditional uses before they are lost forever. (Morgenstern, 2003)

1.2 Medicinal Plants and herbs

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plant derivatives consider as

important source for active ingredients which are used in aspirin and toothpaste. (Hassan, 2012)

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

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

Traditional systems of medicine continue to be widely practiced 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 civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care

needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

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

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

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

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

In several parts of the world many herbs are used to 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. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.

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

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

Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants. (Zahid,2016)

1.3 Historical review on medicinal plants

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants use were instinctive, as is the case with animals. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience.

In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the

advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis.Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again.

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Theae folium*, *Podophyllum*, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra.

The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc.

The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc.

According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense.

In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (*Inula helenium* L. Asteraceae) was named in honor of Elena, who was the centre of the Trojan War. As regards the plants from the genus *Artemisia*, which were believed to restore strength and protect health, their name was derived from the Greek word *artemis*, meaning "healthy."Herodotus (500 BC)

referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (*Scilla maritima*), mustard, and cabbage.

The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (*Centaurium umbellatum Gilib*) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents.

Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. In the description of the plant toxic action, The ophrast underscored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants.

In his work "*De re medica*" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc.

In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic *materia medica* until the late Middle Ages and the Renaissance.Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect. In addition to the plant description, the names in other languages coupled with the localities where they occur

or are grown are provided. The plants having mild effect are dominant, but there are also references to those containing alkaloid or other matter with strong effect (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade). Dioscorides' most appreciated domestic plants are as follows: willow, camomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore). Camomile (*Matricaria recucita* L.), known under the name Chamaemelon, is used as an antiphlogistic to cure wounds, stings, burns, and ulcers, then for cleansing and rinsing the eyes, ears, nose, and mouth. Owing to its mild carminative action, it is particularly appropriate for usage with children. Dioscorides deemed that it had abortive action, on which he wrote, "The flower, root, and the entire plant accelerate menstruation, the release of the embryo, and the discharge of urine and stone, provided that they are used in the form of an infusion and baths." This untrue belief was later embraced by both the Romans and the Arabs; hence the Latin name *Matricaria*, derived from two words: *mater* denoting "mother," i.e. matrix, denoting 'uterus'. Dioscorides differentiated between a number of species from the genus *Mentha*, which were grown and used to relieve headache and stomach ache. The bulbs of sea onion and parsley were utilized as diuretics, oak bark was used for gynaecological purposes, while white willow was used as an antipyretic. As maintained by Dioscorides, *Scillae bulbus* was also applied as an expectorant, cardiac stimulant, and antihydrotic. It is worth underscoring that Dioscorides pointed to the possibility of forgery of drugs, both the domestic ones such as opium forged by a yellow poppy (*Glaucium flavum*) milk sap and poppy, and the more expensive oriental drugs, transported by the Arab merchants from the Far East, such as iris, calamus, caradmomum, incense, etc.

The Arabs introduced numerous new plants in pharmacotherapy, mostly from India, a country they used to have trade relations with, whereas the majority of the plants were with real medicinal value, and they have persisted in all pharmacopoeias in the world till today. The Arabs used aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna, and so forth. Certain drugs with strong action were replaced by drugs with mild action, for instance, *Sennae folium* was used as a mild laxative, compared to the purgatives *Heleborus odorus* and *Euphorbium* used until then.

Throughout the Middle Ages European physicians consulted the Arab works "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980-1037), and "Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248), in which over 1000 medicinal plants were described.

While the old peoples used medicinal plants primarily as simple pharmaceutical forms—infusions, decoctions and macerations—in the Middle Ages, and in particular between 16th and 18th centuries, the demand for compound drugs was increasing. The compound drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug the riac was produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively.

In 18th century, in his work *Species Plantarium* (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. The species were described and named without taking into consideration whether some of them had previously been described somewhere. For the naming, a polynomial system was employed where the first word denoted the genus while the remaining polynomial phrase explained other features of the plant (e.g. the willow Clusius was named *Salix pumila angustifolia antera*). Linnaeus altered the naming system into a binominal one. The name of each species consisted of the genus name, with an initial capital letter, and the species name, with an initial small letter.

Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipeca cuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy. With the upgrading of the chemical methods, other active substances from medicinal plants were also discovered such as tannins, saponosides, etheric oils, vitamins, hormones, etc.

In late 19th and early 20th centuries, there was a great danger of elimination of medicinal plants from therapy. Many authors wrote that drugs obtained from them had many shortcomings due to the destructive action of enzymes, which cause fundamental changes during the process of medicinal plants drying, i.e. medicinal plants' healing action depends on the mode of drying. In 19th century, therapeutics,

alkaloids, and glycosides isolated in pure form were increasingly supplanting the drugs from which they had been isolated. Nevertheless, it was soon ascertained that although the action of pure alkaloids was faster, the action of alkaloid drugs was full and long-lasting. In early 20th century, stabilization methods for fresh medicinal plants were proposed, especially the ones with labile medicinal components. Besides, much effort was invested in study of the conditions of manufacturing and cultivation of medicinal plants.

In present days, almost all pharmacopoeias in the world—Ph Eur 6 USP XXXI,BP 2007—proscribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Plant drugs and phyto preparations, most commonly with defined active components, verified action and, sometimes, therapeutic efficiency, are applied as therapeutic means. In the major European producer and consumer of herbal preparations—Germany, rational phyto therapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs.(Petrovska,2012)

1.4 Importance of medicinal plant

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research

in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs.

Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples.

The efficacy of some herbal products is beyond doubt, the most recent examples being *Silybum marianum* (silymarin), *Artemisia annua* (artemesinin) and *Taxus baccata* (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, *Ginkgo biloba* for tinnitus, *Hypericum perforatum* is a reputed remedy for depression. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb.

Recently research has supported biological activities of some medicinal herbs. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease. *Allamanda cathratica* [allamandin], *Elephatopus elatus* [elephantpoin], *Helenium autmnale* [helenalin] *Vernonia hymenlepis*, *Heliotropium indicum* [Indicine-N-oxide], *Daphne mezereum* (mezerien) and *Stereospermum suaveolans* [laphacol] are medicinal plants that have shown significant tumor inhibiting effect.

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

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

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

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

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

The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces through consideration of primary and secondary metabolites derived as a result of

plant metabolism. The plant material is subjected to phytochemical screening for the detection of various plant constituents.

With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60%of synthetic medicines owe their origin to plants.(Martinez and Staba,2005)

1.5 History of Use of Traditional Herbal Medicines

By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. An example is the use of ephedra (= Ma huang) for weight loss or athletic performance enhancement. While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products are sold as 'phytomedicines', they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the marketplace are marketed and regulated as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria. (Shaw, 1998)

1.5.1 The role of herbal medicines in traditional healing

The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. (Schulz *et al*., 2001)

1.5.2 Traditional Chinese medicine

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12 000 items used by traditional healers, about 500 are in common use 3Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy.

Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000)

1.5.3 Japanese traditional medicine

Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century (Saito, 2000).

1.5.4 Indian traditional medicine

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

1.5.5 Introduction of traditional herbal medicines into Europe, the USA and other developed countries

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines particularly in Europe and North America, where herbal products have been incorporated into so-called ‘alternative’, ‘complementary’, ‘holistic’ or ‘integrative’ medical systems. (Tyler, 2000)

During the latter part of the twentieth century, increasing interest in self-care resulted in an enormous growth in popularity of traditional healing modalities, including the use of herbal remedies; this has been particularly true in the USA. Consumers have reported positive attitudes towards these products, in large part because they believe

them to be of 'natural' rather than 'synthetic' origin, they believe that such products are more likely to be safe than are drugs, they are considered part of a healthy lifestyle, and they can help to avoid unnecessary contact with conventional 'western' medicine.

While centuries of use in traditional settings can be used as testimony that a particular herbal ingredient is effective or safe, several problems must be addressed as these ingredients are incorporated into modern practice.

One problem is that ingredients once used for symptomatic management in traditional healing are now used in developed countries as part of health promotion or disease prevention strategies; thus, acute treatment has been replaced by chronic exposure .This means that a statement about 'thousands of years of evidence that a product is safe' may not be valid for the way the product is now being used. This does not expressly mean that an ingredient is unsafe; it does mean that safety in the modern context cannot be assumed.

A second problem is that efficacy and effectiveness have rarely been demonstrated using modern scientific investigations. An evidence-based approach to this issue has only recently been implemented, and the results reveal that for most herbal products, considerable gaps in knowledge need to be remedied before one can be convinced about their efficacy.

One of the most difficult issues to contend with in translating traditional herbal practices into conventional 'western' medicine is the individualization of prescriptions containing multiple herbal and other ingredients. There is little incentive for standardization of products for a mass market, when the intention has been to provide an individual prescription. To the small grower or the traditionally trained herbalist, standardization means understanding the growth conditions, the time of harvesting, the manner of extraction or other preparation of material so that a reliable (albeit small amount of)active ingredient can be offered to people.

To the manufacturer or distributor of large quantities that will be sold in a supermarket or a health food store, standardization refers to industrial production under defined conditions, using so-called Good Manufacturing Practices (GMP) (Food & Drug Administration, 2002) akin to those used for drug production.

In the USA, there is both small-scale and large-scale production of herbal products and there can be wide variation in their content and quality in the marketplace. Regulations in the USA do not yet require that dietary supplement manufacturers adhere to standard manufacturing practices, and so quality is not guaranteed. The public becomes discouraged by reports that products taken from store shelves do not consistently contain the ingredients or in the amounts that are claimed on the label. For herbal products in common use, evidence of efficacy may be based upon traditional use, testimonials, clinical studies, both controlled and uncontrolled, and randomized, double-blind, placebo-controlled trials.

For the most part, however, there is a lack of systematic clinical studies to support claims. Safety of some herbal ingredients has been recently called into question, in part because of the identification of adverse events associated with their use and, increasingly, because of the demonstration of clinically relevant interactions between herbs and prescription drugs. Adverse events (stroke, heart attacks, heart-rate irregularities, liver toxicity, seizures, psychoses and death) associated with use of ephedra for weight loss, body-building effects and increased energy or kava-kava (also known as kawa), widely used in Europe and increasingly in Canada to treat anxiety, nervousness, insomnia, pain and muscle tension.

1.6 Herbal medicinal products

According to Council Directive 65/65/EEC (European Commission, 1965), which has been implemented in national law in all Member States, medicinal products require prior marketing approval before gaining access to the market. In almost all Member States, herbal medicinal products are considered as medicinal products, and are, in principle, subject to the general regulations for medicines as laid down in the various national medicine laws. In many cases, a specific definition of herbal medicinal products is available, which is in line with the EU Guideline 'Quality of Herbal Medicinal Products'. This includes plants, parts of plants and their preparations, mostly presented with therapeutic or prophylactic claims. Different categories of medicinal products containing plant preparations exist or are in the process of being created. For instance, draft legislation in Spain includes the definitions 'herbal medicinal products' and 'phyto traditional products'. The latter are not considered as

'pharmaceutical specialties' and are therefore not classified as herbal medicinal products.

1.6.1 Classification of herbal products

Generally, herbal products are classified as medicinal products if they claim therapeutic or prophylactic indication, and are not considered as medicinal products when they do not make these claims. Products not classified as medicinal in most cases belong to the food or cosmetic areas, although they sometimes contain plants which have pharmacological properties. For example, senna pods (from *Cassia* plants, used as laxatives) (see General Remarks and monograph on *Rubia tinctorum*, *Morinda officinalis* and anthraquinones in this volume) can be marketed as food in Belgium. Specific categories of non-medicinal products exist in some Member States, such as the so-called 'therapeutic supplement products' in Austria. In Ireland, Spain and the United Kingdom, there exist preparations defined as medicinal products, which are under specific conditions exempt from licensing requirements.

1.6.2 Combination products

Herbal ingredients used in combination are widely used in Europe, and their assessment is often performed according to specific guidelines. Combinations of herbal and homeopathic ingredients exist in a few countries. Their assessment follows rather strict criteria, usually those of a 'full' application procedure. Combinations of herbal ingredients and vitamins are available in many countries. (IARC, 2002)

1.6.3 Importance of Herbal Medicines

Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, bark, etc. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. Herbal remedies can also be purchased in the form of pills, capsules or powders, or in more concentrated liquid forms called extracts and tinctures. They can apply topically in creams or ointments, soaked into cloths and used as compresses, or applied directly to the skin as poultices.

A combination therapy integrating ayurveda and allopathy whereby the side effects and undesirable reactions could be controlled can be thought of. Studies can show that

the toxic effects of radiations and chemotherapy in cancer treatment could be reduced by Ayurvedic medications and similarly surgical wound healing could be accelerated by Ayurvedic medicines. Modern science and technology have an essential role to play in the process. (Zahid,2016)

1.6.4 Some herbs with their medicinal values

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.

- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.

- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.

- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.

- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as *Chirayta*, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.

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

- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.

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

- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.

- Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.

- Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.

- Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.

- Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.

- Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.

- Certain aromatic plants such as Aloe, Golden seal, Barberry and Chirayata are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.

- Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne, Lal Mirch, Myrrh, Camphor and Guggul.

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

- Honey, turmeric, marshmallow and liquorice can effectively treat a fresh cut and wound. They are termed as vulnerary herbs.(Zahid,2016)

1.7 Alternative Medicine

These days the term "Alternative Medicine" became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively.(Hassan,2012)

1.8 Characteristics of Medicinal Plants

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

-**Synergic medicine**: The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

-**Support of official medicine**: In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

-**Preventive medicine**: It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Hassan, 2012)

1.8.1 Future of Medicinal Plants

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of our National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified. Our group has also isolated several compounds, mainly from edible plant species or plants used as dietary supplements that may act as chemo preventive agents. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds. (Balunas and Kinghorn, 2005)

1.8.2 Medicinal plant in Bangladesh

In an estimate, the international market of medicinal plants related to trade stood at 60 billion US Dollar per year. The demand for medicinal plants based raw materials are growing at an approximate rate of 10-15% per year internationally. Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jumping in volume of plants materials trade within and across the country. In Bangladesh there are no systematic cultivation process or conservation strategies about medicinal plants. The local people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation. This knowledge is now under threat to extinction. This is a very alarming situation with regard to natural growth of medicinal plants in the wilderness in this country. In a survey on ―Traditional and industrial use and market Scenario of Medicinal plants in Bangladesh.‖ conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh, found that there is worth

of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products. Another research aimed at documenting the Present Status and Market Scenario of Medicinal Plants‘ in Bangladesh shows that 84.1% of the respondent use medicinal plants in health care. 18.3% of the villagers use Kabirazi in the disease in medium category. 55.0% of respondent‘s source of knowledge of using medicinal plant is family where 34.7% gained knowledge from neighbor. Only 14.3% of the respondents are involved with trading of medicinal plant. About 10.4% of the villagers are involved in cultivation, collection or business of medicinal plant. From the survey report it has been found that 46.6% industries are using above 60% of imported medicinal plants as their raw materials and 53.3% of the industries are using below 40%. The study revealed that 86.7% industries are importing Indian raw materials, 53.3% are importing the Pakistani one and very few of them are importing the raw materials from Nepal, Iran and Korea. According to the response of shop owners, the local raw materials of their products are mostly coming from 5 different areas of the country. Among those 90% are coming from Chittagong and again 76.6% from Tangail, 30% from Gazipur and another 30% from Khulna. In this scenario, appropriate steps must therefore be taken immediately in order to save this situation with regard to growth, conservation and supply of medicinal plants in the country. (Mpbd.info, 2014)

List of medicinal plant in Bangladesh and their uses

SL No	**Local Name**	**Scientific Name**	**Family**	**Using part**	**Control Disease Name**	Image of the Plant
1	Neem	*Azadirachta indica*	Meliaceae	Root, leaf Bark	Skin disease, worm killer Arthritis, Insecticide, Jaundice etc. Antiviral.	
2	Bel	*Aegle marmelos*	*Aegle marmelos*	Fruit	Dysentery Diarrhea.	
3	Durba	*Cynodon dactylon*	Grominae	Leaf	Blood bleeding control. Skin Disease.	
4	Grhitokumari	*Aloe indica*	Liliaceae	Extract of leaf	Headache, sexual disease, Metabolic problem. Fever.	

5	Pudina	*Mentha viridis*	Lebiatae	Whole plant	Metabolic disorder, Gastric.	
6	Basok	*Adhatoda vasica*	Acanthaceace	Leaf, root of plant	Cough, asthma, tuberculosis, Cold, blood refine.	
7	Joshti Madhu	*Hydrangea arborescons*	Saxifra zaceac	Leaf flower fruit	Liver disease, adrenal peptic Ulcer, hormonal disease, cold, Throat pain.	
8	Sharpo gandha	*Rauvolfia Serpentina*	Apocynaceae	Leaf and root.	Blood Pressure, brain abnormal, dysentery diarrhea Pain killer.	

9	Brami shak	*Becopa moniera*	Scrophulariaceae	Leaf	Heart disease, nurval pressure, Asthma.	
10	Kurci	*Holarrhena antidysenterica*	Apocynaceae	Bark & Seed	Diarrhea, dysentery, worm killer constipation, intestinal weakness	
11	Kal megh	*Andrographs pariculata*	Acanthaceae	whole plant	Metabolic problem, Gastric, Fever, worm killer, Dysentery, Liver Disease, Strengthen.	
12	Akon d	*Calotropis procera*	Asclepiadaceae	Root, leaf. Bark flower extract of Leaf.	Ulcer, Tooth pain chronic dysentery, cold, Asthma	

13	Anantmul	*Hemidesmus indicus*	Asclepia-daceae	Root and whole plant	Strength increaser, apetiser. Arthritis, Diabetes.	
14	Apang	*Achyranthes Paniculata*	Amaranthaceae	whole plant	Dysentery, Constipation, piles, Arthritis, Skin disease.	
15	Arjun	*Terminalia arjuna*	Combretaceae	Bark	Heart disease, Diarrhea, piles, Tuberculosis.	

(Yusuf et al., 2007)

Table 1 : List of medicinal plant in Bangladesh and their uses

1.9 Review of *Garcinia cowa*

1.9.1 General Information

Family: Clusiaceae

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

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

Binomial name: *Garcinia cowa* Roxb

English name: Cow Tree (Uddin,2014)

1.9.2 Taxonomy

Kingdom: Plantae

Subphylum: Euphyllophytina

Infraphylum: Radiatopses

Subclass: Dilleniidae

Superorder: Theanae

Order: Malpighiales

Family: Clusiaceae

Tribe: Garcinieae

Genus: *Garcinia*

Species*: G. cowa* (Hassler,2015)

1.9.3 Different species of *Garcinia*

- *Garcinia cambogia*
- *Garcinia indica*
- *Garcinia cowa*
- *Garcinia pedunculata*
- *Garcinia kydia*
- *Garcinia lanceaefolia*
- *Garcinia oliveri*
- *Garcinia opaca*
- *Garcinia paucinervis*
- *Garcinia pyrifera*
- *Garcinia quaesita*
- *Garcinia rubro-echinata*
- *Garcinia schomburgkiana*
- *Garcinia scortechinii*
- *Garcinia semseii*
- *Garcinia heterandra*
- *Garcinia holttumii*
- *Garcinia maingayi*
- *Garcinia mannii*
- *Garcinia mangostana – purple mangosteen*
- *Garcinia mestonii*
- *Garcinia minutiflora*
- *Garcinia monantha*
- *Garcinia zeylanica*
- *Garcinia montana* (Cheek,2004)

1.9.4 Plant description

The Genus *Garcinia*, belonging to the Family Clusiaceae which comprises about 300 species, have been widely investigated in terms of their bioactive ingredients. Native to Asia, Africa, South America and Polynesia, the plants are small to medium sized

evergreen trees which may grow up to 30 m in height and are widely distributed in the tropical and temperate regions of the world .Twenty-nine species have been observed in Thailand, with 20, 13, 12, 7, 6 and 3 species found in the south, middle, north, east, north-east and west of the country respectively.

Garcinia cowa is an abundant source of bioactive phytochemicals. Phytochemical investigations of the plant parts indicated that the fruit, twig and stem are the best source of secondary metabolites, providing flavonoids, phloroglucinols and xanthones respectively. Seventy eight of these compounds have been identified from the plant and several have interesting pharmacological activities.

Garcinia is a rich source of secondary metabolites, especially triterpenes, flavonoids, xanthones and phloroglucinols. The latter two groups are well recognized as cheomotaxonomic markers for this genus. Many of the isolated compounds have a wide range of pharmacological activities including anticancer, antiinflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant and antioxidant.

Garcinia cowa commonly known as Cha-muang in Thai, is widely distributed throughout Malaysia, Thailand and Myanmar. The fruits and young leaves are edible with a sour taste. The bark is dark brown with a yellow latex.The plant has unisex flowers: yellow orange female flowers found at the end of branches and male flowers found along the branches as clusters.The leaves are glossy, deep green, oblong and up to 6-15 cm in length and 2.5-6.0 cm in width .The fruits are globose (2.5-6.0 cm in size), green when young and dull orange or yellow at maturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3-angled seeds.

Figure 1*: Garcinia cowa* **plant**

1.9.5 Different parts of *Garcinia cowa*

Many parts of *G. cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an antifever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant. The chemical composition and biological activities of various parts of *G. cowa* have been investigated. The major compounds found were xanthones and phloroglucinols. However, minor compounds, including depsidones, terpenoids, steroids and flavonoids, were also observed. Currently, 78 compounds have been isolated from the twig ,stem ,fruit and latex.(Ritthiwigrom et al.,2013)

Figure 2: Different parts of *Garcinia cowa*

1.9.6 Uses:

- Bark is astringent; used in spasm. Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery.
- Gum resin is drastic cathartic, may produce nausea and vomiting.
- Ethanolic extract of the leaf possesses antibacterial properties.
- Ripe fruits are edible, sour in taste, uncomfortable feeling in the mouth due to stick juice (Chakma).
- Ripe fruits are eaten, sour in taste (Khumi).
- Fruit is eaten when the dog is beaten by snake; the affected dog placed in a piece of leaves and also covered with leaves as the treatment (Murang).
- Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery (Tripura).
- Exercise performance. Taking a chemical compound found in Garcinia called hydroxyl citric acid (HCA) might increase how long untrained women are able

to exercise. However, it does not seem benefit men in the same way.(Uddin,2014)

1.9.7 Distribution and biological activity

The biological activities of the extracts from various parts of *G. cowa* have been investigated, including the hexane and chloroform extracts of the fruit rind and methanol extract of the leaves and twigs. The hexane and chloroform extracts from the fruit rind of *G. cowa* were tested against four Gram-positive bacteria (*Bacillus cereus*, *B. coagulans*, *B. subtilis* and *Staphylococcus aureus*) and one Gram-negative bacterium (*Escherichia coli*).Both extracts significantly inhibited bacterial growth of the Gram-positive bacteria (IC50s 15-30g/mL) but not *E. coli* (IC_{50}s 250-500g/mL) The extracts were also found to inhibit the growth of *Aspergillus flavus* ATCC 46283, a common fungal food contaminant which produces aflatoxin B1.The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (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 per oxidation induced by copper ions. The twig extract had an IC50 value of 20.5 g/mL and was more potent (higher % inhibition at 1000g/mL) than the leaf extract (IC_{50} not measured). The twig extract was more potent than the leaf extract on platelet aggregation of human whole blood induced by arachidonic acid, adenosine diphosphate and collagen. These activities may be due to the total phenolic content of these extracts, which were 19 and 61 mg of Gallic Acid equivalent per g of extract for the leaf and twig extracts respectively.

1.9.8 Classes of chemical compounds isolated from *Garcinia* Cowa

1.9.8.1 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 (11) and morelloflavone-7□□-O-glucoside (13) showed strong antioxidant activities.

1.9.8.2 Phloroglucinols

Phloroglucinols are based on a phloroglucinol or 1,3,5-benzenetriol core skeleton or its 1,3,5-cyclohexanetrione (phloroglucin) tautomer. The phloroglucinols found in G. cowa have a benzoyl group and geranyl and polyprenyl units as substituent groups. So far, fifteen phloroglucinols have been obtained from the twig including six new compounds: guttiferone K (15a), chamuangone (16), garcicowins A (17), B (18), C (21) and D (22) and nine known phloroglucinols: cambogin (14), guttiferones K (15b), B (25) and F(26), oblongifolins B (19), C (20), A (24) and D (27), and 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.

1.9.8.3 Terpenes and Steroids

Terpenes and steroids represent two large classes of natural products, although they are rare in G. cowa. Only four of these types of compounds (5% of the total compounds isolated) were present in *G. cowa*, viz. friedelin (28), daucosterol (29),-sitosterol (30) and stigmasterol (31). None of these compounds were further studied for their biological activities.

1.9.8.4 Xanthones

Xanthones, with two aromatic rings linked via carbonyl and ether linkages, are the major components of the Garcinia genus [8c-e]. They are commonly found in several parts of *G. cowa*, especially in the stem, fruit and latex. Thirty six xanthones (46% of the total isolated compounds) have been isolated and nineteen of them were first isolated from *G. cowa*. They are cowagarcinone C (32), cowaxanthone (43), cowanol (45), cowanin (46), 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone (47), norcowanin (48), cowagarcinones A (49), B (50), E (51) and D (52) from the latex [15, 30]; cowaxanthones B (34), C (39), D (42) and E (44) from the fruit ; 7-O-methylgarcinone E (36), 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl) xanthone (59), 4-(1,1-dimethyl-prop-2-enyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl) xanthen-9(9H)-one (61) and 1,5-dihydroxy-3methoxy-6′,6′-

dimethyl-2H-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone(62) from the stem and cowaxanthone F (55) from the twig .Most of these xanthones showed interesting biological activities.(Ritthiwigrom, T.,2013)

1.9.8.5 Depsidone

Depsidones comprise benzoic acid and phenol skeletons condensed at the ortho positions through ester and ether linkages. This class of natural products is well known in the *Garcinia* species. However, cowadepsidone (1) was the first and only known depsidone from *G. cowa*. It was isolated from the twig extract and showed cytotoxicity against NCI-H187 and MFC-7 cancer cell lines

1.9.8.6 Miscellaneous Compounds

Ten (13% of the total isolated compounds) of the miscellaneous class of compounds have been isolated, including a new discovery: (2E,6E,10E)-(+) -4β-hydroxy -3-methyl5 β-(3,7,11,15-tetramethyl-hexadeca-2,6,10,14-tetraenyl)cyclohex-2- en -1-one (68) None of the isolated compounds from this class were tested for their biological activities.(Ritthiwigrom et al.,2013)

1.9.9 Biological activities of Chemical constituent (Xanthone) of *Garcinia cowa* Roxb

1.9.9.1 Antibacterial activity:

Eight xanthones from the fruit: cowaxanthones B (34) and C (39), 7-O-methylgarcinone E (36), α-mangostin (37), β-mangostin (38), mangostanin (40), cowanol (45) and cowanin (46) were investigated for their antibacterial activity against S. aureus and MRSA. α-Mangostin (37) and mangostanin (40) showed significant activity against these bacteria. α-Mangostin (37) had a MIC value of 8 µg/mL against both *S. aureus* and MRSA while mangostanin (40) had an MIC value of 4 µg/mL against both bacteria.

1.9.9.2 Anti-inflammatory activity :

Eight xanthones: cowaxanthones A (32), B (34), C (39) and D (42), α-mangostin (37), mangostanin (40), cowanol (45) and cowanin (46) were tested for their anti-inflammatory activity using the ethyl phenylpropiolate induced ear edema assay. All xanthones except cowanol were more active than the standard drug, phenylbutazone.

1.9.9.3 Antimalarial activity:

Five xanthones isolated from the stem bark: 7-O-methylgarcinone E (36), α-mangostin (37), cowaxanthone (43), cowanol (45) and cowanin (46) had significant in vitro antimalarial activity against Plasmodium falciparum with IC50 values ranging between 1.5-3.0 µg/mL.

1.9.9.4 Anticancer activity:

Six xanthones: cowaxanthone (43), cowanol (45), cowanin (46), norcowanin (48), 3,6-di-Omethyl-γ-mangostin (57) and dulxanthone A (60) isolated from twig were evaluated for their cytotoxicity against NCI-H187, KB, MFC-7 and/or HepG2 cell lines. Cowaxanthone (43), cowanin (46), norcowanin (48) and 3,6-di-O-methyl-γ-mangostin (57) exhibited significant cytotoxicity against the NCI-H187 cell line with IC_{50} values ranging between 3.87-8.58 µg/mL, and moderately inhibited KB and MCF-7 cancer cell lines with IC_{50} values ranging between 6.43-15.43 and 10.59-21.38 µg/mL respectively. Dulxanthone A (60) was found to be cytotoxic against the HepG2 cell line .(Ritthiwigrom et al.,2013)

1.10 Antioxidants

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include-

- Beta-carotene
-Lutein
- Lycopene
- Selenium

-Vitamin A
-Vitamin C
-Vitamin E

Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines. To minimize risk, tell you of your health care providers about any antioxidants you use. (U.S. National Library of Medicine, 2017)

1.10.1 Classification of Antioxidants

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

1.10.1.1 Enzymatic Antioxidants:

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

1.10.1.1.1 Primary Antioxidants:

Primary antioxidants mainly include super oxide dismutase (SOD),catalase (CAT), and glutathione per oxidase (Gpx).

Super oxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O−) and repairs the body cells damaged by free

radical. SOD catalyzes the reduction of super oxide anions to hydrogen peroxide. SOD is also known to compete with nitric oxide (NO) for super oxide anion, which inactivates NO to form peroxy nitrite. Therefore, by scavenging super oxide anions, it promotes the activity of NO.

Catalase enzyme (CAT) is found in the blood and most of the living cells and decomposes H_2O_2 into water and oxygen. Catalase with glucose peroxidase is also used commercially for the preservation of the fruit juices, cream consisting of egg yolk, and salad by removing the oxygen.

Glutathione per oxidase (GPx) is a group of selenium dependent enzymes, and it consists of cytosolic, plasma, phospholipid hydro per oxide, and gastrointestinal glutathione per oxidase. GPx (cellular and plasma) catalyzes the reaction of H_2O_2 by reduced glutathione (GSH); as a result, oxidized glutathione (GSSG) is produced and it is again recycled to its reduced form by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

1.10.1.1.2 Secondary Antioxidant:

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

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), 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.

1.10.1.2 Non enzymatic Antioxidants

They are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism. Some of the known non enzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants.

1.10.1.2.1 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 radish-orange-green color food items including carrots, sweet potatoes, apricots, pumpkin, mangoes, and cantaloupe along with some green and leafy vegetables, including collard greens, spinach, and kale. Lutein is abundant in green leafy vegetables such as collard greens, spinach, and kale. Lutein is best known for its role in protection of retina against harmful action of free radicals and also prevents atherosclerosis. Although lycopene, lutein, canthaxanthin, and zeaxanthin do not possess provitamin A activity, β-carotene is known as a precursor for vitamin A. 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.

1.10.1.2.2 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. These consist of phenolic acids, flavonoids, gingerol, curcumin etc. 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 (diferuloyl methane) is the main bioactive component of turmeric and is known to possess good antioxidant activity. Curcumin is an excellent scavenger of ROS, such as O_2 radicals, lipid peroxyl radicals (LO_2), OH radicals, and nitrogen dioxide (NO_2) radicals, which induced oxidative stress. Curcumin has been shown to inhibit lipid per oxidation and has been shown to increase GSH levels also in epithelial cells which lead to lower ROS production.

1.10.1.3 Other Antioxidants:

1.10.1.3.1 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.

1.10.1.3.2 Non protein Antioxidants:

Bilirubin, uric acids, and ubiquinol are non protein antioxidants which inhibit the oxidation processes by scavenging free radicals. Bilirubin is an end product of heme catabolism. It is a lipid-soluble cytotoxic product that needs to be excreted. However, bilirubin efficiently scavenges peroxyl radical at micromolar concentrations in vitro model and is regarded as the best antioxidant against lipid per oxidation. (Academic library, 2014)

2.1 Organic Acids from Leaves, Fruits, and Rinds of *Garcinia cowa*

Organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa* (*G. cowa*) were determined by high-performance liquid chromatography. Fresh leaves, fruits, and dried rinds were extracted with water at 120 °C for 20−30 min under 15 lbs/in^2 pressure. Also, dried rinds were extracted with solvents (acetone and methanol) using a Soxlet 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 (−)-hydroxy citric acid present in leaves, fruits, and rinds to the extent of 1.7, 2.3, and 12.7%, respectively. (−)-Hydroxy citric 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* (Bhabani et al., 2002)

Figure 3: Leaves of *G. cowa*

Figure 4: Ripe fruits of *G. cowa*

2.2 The Constituents from the Stems of *Garcinia cowa* Roxb. and their cytotoxic activities

Three new flavanone glycosides named garccowaside A, garccowaside B, garccowaside C, and three other known compounds were isolated from the ethanol extract of the stems of *Garcinia cowa*. These structures were established on the basis of spectroscopic evidence. Twelve compounds isolated from the stems of *Garcinia cowa* were tested for cytotoxic activities. (Shen et al., 2007)

Figure 5: Stem of *Garcinia cowa*

2.3 The Use of *Garcinia* Extract (Hydroxy citric Acid) as a Weight loss Supplement: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

Obesity is one of the pandemic chronic diseases commonly associated with health disorders such as heart attack, high blood pressure, diabetes or even cancer. Among the current natural products for obesity and weight control, *Garcinia* or more specifically hydroxyl citric acid (HCA) extracted from *Garcinia* has been widely used. The evaluation of the potential toxicity of weight control supplement is of the utmost importance as it requires long term continuous consumption in order to maintain its effects. Majority of reports demonstrated the efficacy of *Garcinia*/HCA without any toxicity found. However, a few clinical toxicity reports on weight-loss diet supplements of which some were combinations that included *Garcinia*/HCA as an active ingredient showed potential toxicity towards spermatogenesis. Never the less, it cannot be concluded that *Garcinia*/HCA is unsafe. Those products which have been reported to possess adverse effects are either polyherbal or multi-component in nature. To date, there is no case study or report showing the direct adverse effect of HCA. The structure, mechanism of action, long history of the use of *Garcinia*/HCA and comprehensive scientific evidence had shown "no observed adverse effect level (NOAEL)" at levels up to 2800 mg/day, suggesting its safety for use. (Chuah et al., 2012)

2.4 Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* Oliver (Clusiaceae)

The methanolic extract (GSM) prepared from the stem bark of *Garcinia smeathmannii* as well as ten compounds isolated from this crude extract, were tested for their antimicrobial activity against Gram-positive bacteria (6 species), Gram-negative bacteria (12 species) and 3 Candida species using well micro-dilution methods. The GSM showed very interesting inhibition effects on the growth of the tested pathogens with the minimal inhibition concentrations (MIC) lower than 156.25 µg/mL on 21 of the 22 pathogens tested. Purified compounds showed selective activities. Two of these compounds namely Cheffou xanthone (**1**) and Friedelin (**9**) exhibited both antibacterial and anticandidal activities. The antimicrobial activity of compounds **1**, Bangang xanthone A (**4**), and Guttiferone I (**7**), as well as that of GSM is being reported for the first time. The overall results provide promising baseline information for the potential use of the crude extract from the stem bark of *G. smeathmannii* as well as some of the isolated compounds in the treatment of bacterial and fungal infections. (Kuete et al., 2007)

Figure 6: Branch and bark of *Garcinia cowa*

2.5 Microencapsulation of *Garcinia Cowa* Fruit Extract and effect of its use on Pasta Process and Quality

Microencapsulation is employed to protect bioactive ingredients in foods and is also used for their controlled release at targeted sites. Hydroxy citric acid ((-)-HCA) is present in the fruits of certain species of *Garcinia* and it has been studied extensively

for its unique regulatory effect on fatty acid synthesis, lipogenesis, appetite, and weight loss. Since hydroxyl citric acid is hygroscopic in nature, it is very difficult to convert liquid extract from the fruits of *Garcinia* into dried powder. Hence, microencapsulation of *Garcinia cowa* fruit extract was performed in a pilot-scale co-current spray dryer with whey protein isolate as a wall material. In this study, two different wall-to-core ratios (1:1 and 1.5:1) and dryer outlet temperatures (90 and 105°C) were used for assessing the encapsulation efficiency. The results in this study showed that the microencapsulation efficiency (based on HPLC analysis) and antioxidant properties (based on 2,2-diphenyl-1-picrylhydrazyl assay) were higher at 90°C outlet temperature of the spray dryer using 1.5:1 wall-to-core ratio feed. Further, the spray-dried powders were incorporated into pasta processing and evaluated its quality characteristics. The results of this study demonstrated that incorporation of powder spray-dried at 90°C outlet temperature with 1.5:1 wall-to-core pasta exhibited higher antioxidant activity as well as better cooking and sensory characteristics.(Pillai et al.,2012)

2.6 Evaluation of Antioxidant and Antimutagenic Activities of the Extracts from the Fruit Rinds of *Garcinia cowa*

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 hydroxyl anisole (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. (Negi et al., 2010)

Figure 7: Fruit of *Garcinia cowa*

2.7 Cryopreservation of *Garcinia cowa* shoot tips by vitrification: The effects of sucrose preculture and loading treatment on ultrastructural changes in meristematic cells

The effects of sucrose preculture duration and loading treatment on tolerance of *Garcinia cowa* shoot tips to cryopreservation using the PVS2 vitrification solution were investigated. Ultrastructural changes in meristematic cells at the end of the preculture and loading steps were followed in an attempt to understand the effects of these treatments on structural changes in cell membranes and organelles. Increasing preculture duration on 0.3 M sucrose medium from 0 to 3 days enhanced tolerance to PVS2 solution from 5.6% (no preculture) to 49.2% (3-day preculture). However, no survival was observed after cryopreservation. Examination of meristematic cells by transmission electron microscopy revealed the progressive accumulation of an electron-dense substance in line with increasing exposure durations to 0.3 M sucrose pre culture. Treatment with a loading solution (2 M glycerol + 0.4 M sucrose) decreased tolerance of shoot tips to PVS2 vitrification solution and had a deleterious effect on the ultrastructure of *G. cowa* meristematic cells. This study suggests that *G. cowa* meristematic cells may lose their structural integrity due to exposure to glycerol present in the loading solution at a 2 M concentration, either due to its high osmotic potential, or due to its cytotoxicity. (Yap et al., 2011)

2.8 A New Prenylated Xanthone from Latex of *Garcinia cowa* Roxb.

A new Prenylated Xanthone,1,6-dihydroxy-3,7-dimethoxy-2-(3,7-dimethyloct-2,6-dienyl) xanthone (3-O-methylcowaxanthone) (1), together with four known xanthones, cowaxanthone(2),7-O-methylgarcinone(3),α-mangostin (4) and γ-mangostin (5) were isolated from the latex of *Garcinia cowa*. The structure of compound 1was elucidated on the basis of spectroscopic data interpretation, including 1D and 2D NMR and HREIMS. The cytotoxic activity of 1against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7 and SW480, was evaluated, but it was inactive (IC50>40μM) (Na et al., 2013)

2.9 Cowaxanthone F, a new tetra oxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*

A new tetra oxygenated xanthone, cowaxanthone F (1), as well as four known compounds, morelloflavone (2), volkensiflavone (3), morelloflavone-7 "-O-glucoside (fukugiside, 4), and 1,6-dihydroxyxanthone (5), were isolated from the crude acetone extract of the twigs of *Garcinia cowa* (Guttiferae). All compounds (1-5) were tested for antioxidant activity against DPPH (diphenyl picryl hydrazyl), hydroxyl, and superoxide radicals; only morelloflavone (2) and morelloflavone-7 "-O-glucoside(4) exhibited high potency. Eight tetraoxygenated xanthones from the fruits of *G. cowa*, cowaxanthones A-D (6-9), cowanin (15), alpha-mangostin (16), mangostanin (17), and cowanol (18), were also investigated for anti-inflammatory activity using ethyl phenylpropiolate (EPP)-induced car edema. Assessment at 30, 60, and 120 min revealed that cowaxanthones B-D (7-9), cowanin (15), and alpha-mangostin (16) exhibited significant anti-inflammatory activity when compared to phenylbutazone, while cowaxanthone A (6), mangostanin (17),and cowanol (18) showed less activity. Cowaxanthone F, a new tetra oxygenated xanthone, and other anti-inflammatory and antioxidant compounds from Garcinia cowa. (Panthong et al., 2009)

2.10 Cytotoxicity Study of Ethanol Extract of the Leaves of Asam Kandis (*Garcinia cowa* Roxb.) on T47D Breast Cancer Cell line

To investigate the cytotoxic effect of ethanolic extract of the leaves of asam kandis (*Garcinia cowa* Roxb.) against T47D breast cancer cells the cytotoxicity of ethanol

extract was carried out by measuring the activity of mitochondrial dehydrogenase in living cells that have ability to convert dissolved MTT pale yellow to purple for mazan product. The extract was added at various concentrations (0.1, 1, 10 and 100 μg/mL). The level of cytotoxicity was determined by calculating the IC_{50} value that was based on the percentage of the cell death after 24 hours treatment with the extract. Cell morphological changes were observed by using inverted microscope. Results: The IC_{50} value showed that ethanol extract of leaves of asam kandis could resist T47D breast cancer cells with IC_{50} 6.13 ± 3.51 μg/mL. The statistic results proved that ethanol extract of the leaves of asam kandis could inhibit the growth of T47D breast cancer cells significantly at concentrations of 10 μg/mL and 100 μg/mL. The results suggest that ethanol extract of the leaves of asam kandis was potential source of herbal medicine for cancer related ailment. (Husni et al., 2015)

2.11 Cytotoxic Acylphloroglucinol Derivatives from the Twigs of *Garcinia cowa*

An unusual polyprenylated acylphloroglucinol derivative unsubstituted at C-2 and C-6, garcicowin A (1), together with three other new (garcicowins B-D, 2-4) and nine known analogues, was isolated and characterized from the twigs of *Garcinia cowa*. The structures of 1-4 were elucidated by interpretation of their spectroscopic data. The compounds isolated were evaluated for their cytotoxicity against two cancer cell lines (HT-29 and HCT116) and against normal colon cells (CCD-18Co), and the results demonstrated their selective toxicity toward the cancer cell. (Xu et al.,2010)

2.12 Xanthones from the Leaves of *Garcinia cowa* Induce Cell Cycle Arrest, Apoptosis, and Autophagy in Cancer Cells

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

suggested that 17 induced autophagy. Taken together, our results suggest that these xanthones possess anticancer activities targeting cell cycle, apoptosis, and autophagy signaling pathways. (Xia et al., 2015)

Figure 8: Leaves of *Garcinia cowa*

2.13 Tetra oxygenated xanthones from the fruits of *Garcinia cowa*

Tetra oxygenated xanthones, cowa xanthones A–E, together with 10 previously reported tetra oxygenated xanthones, were isolated from the crude hexane extract of the fruits of *Garcinia cowa*. Cowa xanthone B has previously been reported as a synthetic xanthone. Their structures were elucidated by analysis of spectroscopic data, especially by 1D and 2D NMR. The antibacterial activities of the isolated compounds were also evaluated. Tetra oxygenated xanthones: cowa xanthones A–E were isolated from the crude hexane extract of the fruits of *Garcinia cowa*, and the antibacterial activity of some of them investigated. The hexane extract of the fresh fruits of *G. cowa* was subjected to chromatographic purification to yield five new tetra oxygenated xanthones (cowa xanthones A–E: 1, 2, 3, 4 and 5), together with 10 known tetra oxygenated xanthones: l,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2-butenyl) xanthone (6), fusca xanthone C (7), 7-O-methylgarcinone E (8), b-mangostin (9), cowanol (10), mangostanin (11), 6-O-methylmangostanin (12), cowanin (13a-mangostin (14) cowa xanthone (15) All structures were elucidated using 1D and 2D NMR spectroscopic data. The 1 H and/or 13C spectroscopic data of known xanthones were also compared with those reported in the literatures. All new xanthones showed UV absorption bands of xanthone chromophores at kmax 243–246 nm (strong), 258–268 nm (strong), 311–318 nm (medium) and 352–387 nm (weak) while those with a chromene unit conjugated to the xanthone nucleus, i.e. 3 and 4, exhibited

bathochromic shifts of the same absorption bands. All compounds showed IR absorption bands at 3266–3412 and 1631–1649 cm1 for hydroxyl and conjugated carbonyl groups, respectively. In the 1 H NMR spectra, a singlet proton at dH 12.98–14.52 revealed the presence of a hydroxyl group at C-1, chelated to a carbonyl group of the xanthone. A signal of deshielded methylene protons of a prenyl side chain at dH 4.05–4.13 (except for 1 and 4) due to the anisotropic effect of the carbonyl group (C-9) (Panthong et al., 2006)

2.14 Two New Xanthones from the Stems of *Garcinia cowa*

Two new xanthones,1 ,5 ,6 – trihydroxy - 3 -methoxy-4-(3-hydroxyl-3-methylbutyl) xanthone (1) and 1,5-dihydroxy-3-methoxy-6',6'-dimethyl-2H-pyrano (2',3':6,7)-4-(3-methylbut-2-enyl) xanthone (2),have been isolated together with six known xanthones: 1 , 3 , 5 – trihydroxyl - 6' , 6' - dimethyl-2H-pyrano(2',3':6,7) xanthone(3), dulxanthone A (4), 1,5,6-trihydroxy-3,7-dimethoxyxanthone (5),1,7-dihydroxy xanthone(6), 1,3,5-tri hydroxy-6-methoxy xanthone (7),1,3,6,7 tetra hydroxy xanthone (8), from the stems of *Garcinia cowa* (Guttiferae) (Shen and Yang,2006)

3.1Theory of Phytochemical Screening

3.1.1. Materials (Reagents and Tools) Used

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

Table 2: List of reagent used for phytochemical screening

3.1.2 Test Compounds

Methanol extract of stem of *Garcinia cowa*

3.1.3 Preparation of Sample Solution

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

3.1.4 Phytochemical Tests

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

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

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

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

iv. **Borntragers's test for anthraquinone glycosides:** 1 ml of sample solution was shaken with 5 ml of chloroform in a test tube for at least 5 minutes then again shaken with an equal volume of 10% ammonia solution. A bright pink, red or violet color was developed in the aqueous (upper) layer in the presence of free anthraquinones.

v. **Tests for alkaloid:** A small volume of each extract was neutralized by adding 1 or 2 drops of dilute H_2SO_4. This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation was observed.

   a) **Mayer's reagent**: Formation of white and cream color precipitate indicated the presence of alkaloids.

b) **Hager's reagent**: Formation of yellow crystalline precipitate indicated the presence of alkaloids.

c) **Wagner's reagent**: Formation of brownish-black ppt indicated the presence of alkaloids.

d) **Dragendroff's reagent:** Formation of orange or orange-red precipitate indicated the presence of alkaloids.

vi. **Test for saponins:** about 0.5 ml of extract was shaken vigorously with water in a test tube. If a forthing was produced and it was stable for 1-2 minutes and persisted on warming, it was taken as preliminary evidence for the presence of saponins.

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

viii. **Test for steroids:** A small amount of extract was added with 2 ml of chloroform, then 1 ml of conc. H_2SO_4 was carefully added from the side of the test tube. In presence of steroids, a red color was produced in chloroform layer.

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

3.2 Assessment of In Vitro Pharmacological Property

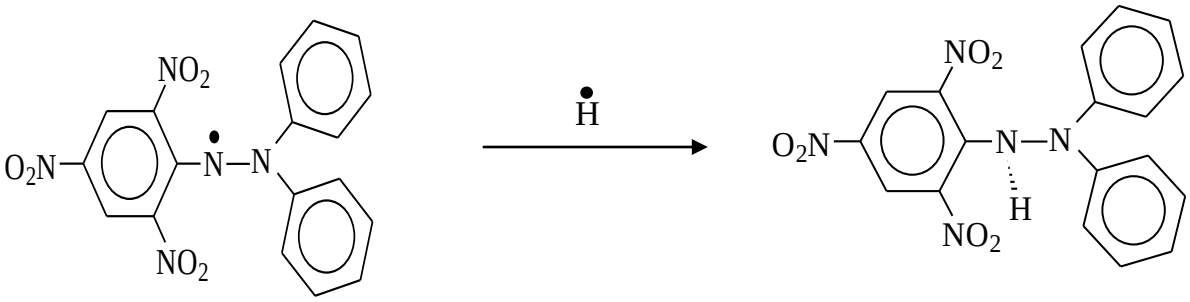
3.2.1 Determination of Antioxidant property

3.2.1.1DPPH Free Radical Scavenging Assay

Principle

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

of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless. (Braca *et al.*, 2001)



1,.1-diphenyl-2-picryl hydrazyl **1,.1-diphenyl-2- picryl hydrazine**

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

Table 3: List of reagent used for DPPH test

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

Preparation of Standard/ Extract solution

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

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

Table 4: Preparation of methanol extract of *G. cowa* stem or ascorbic acid solution

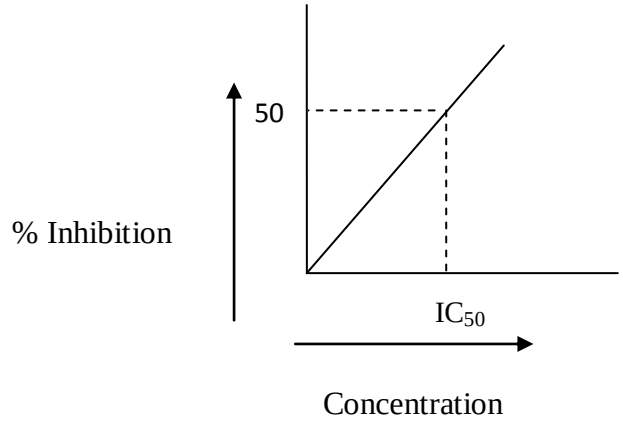
Procedure

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

Calculation

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

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



3.2.1.2 Determination of Total Phenolics Content

Principle

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

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

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

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

Table 5: List of reagent used for total phenol test

Preparation of 7.5% Sodium carbonate solution

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

Preparation of Standard solution

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

Concentration (µg/ml)	Solution taken from stock solution (µl)	Solution taken from others	Adjust the volume by distilled Ethanol (µl)	Final volume (ml)
200	80	-	1920	2
100	-	1 ml (200 µl/ml)	1000	2
50	-	1 ml (100 µl/ml)	1000	2
25	-	1 ml (50 µl/ml)	1000	2
12.5	-	1 ml (25 µl/ml)	1000	2
6.25	-	1 ml (12.5 µl/ml)	1000	2

Table 6: Preparation of Gallic Acid solution

Preparation of Extract solution

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

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

Table 7: Preparation of methanol extract of *Garcinia cowa* stem solution

Experimental Procedure

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

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

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

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

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

3.2.2 Antimicrobial Screening

The antimicrobial activity of the plant extract was performed by the well accepted Bauer-Kirby method (Bauer *et al.*, 1966; Drew *et al.*, 1972).

3.2.2.1 Materials

3.2.2.1.1 Microorganisms

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

3.2.2.1.2 Test Organisms

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

3.2.2.1.3 List of Test Bacteria:

Bacillus sereus	*Vibrio parahemolyticus*
Bacillus megaterium	*Staphylococcus aureus*
Bacillus subtilis	*E.Coli*
Salmonella paratyphi	*Shigella dysenteriae*
Salmonella typhi	*Pseudomonas aureus*

Table 8: List of test bacteria

3.2.2.1.4 Culture Media and Chemicals

- Nutrient agar media

- Ethanol
- Chloroform

3.2.2.1.5 Equipments

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

3.2.2.1.6 Test Materials

The methanolic, extract of *Garcinia cowa* stem were tested against gram-positive and gram-negative bacteria.

3.2.2.2 Methods

3.2.2.2.1 Culture Preparation

Composition of culture media

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

Composition of Nutrient agar media (1000 ml)

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

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

3.2.2.2.2 Sterilization Procedure:

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glass wares were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15 lbs/sq. inch for 20 min. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

3.2.2.2.3 Preparation of Subculture

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

3.2.2.2.4 Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media

3.2.2.2.5 Preparation of Discs

3.2.2.2.5.1 Standard discs

Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Kanamycin (30µg/disc) standard disc was used as the positive control.

3.2.2.2.5.2 Blank discs

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

3.2.2.2.5.3 Preparation of sample discs with test samples

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

3.2.2.2.6 Placement of Disc and Incubation

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

3.2.2.2.7 Determination of Zone of Inhibition

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

4.1 Phytochemical Screening of methanol extract of *Garcinia cowa* stem

Carbohydrate	Alkaloid	Glycoside	Saponin	Steroid	Flavonoid	Tannin
+	-	++	-	++	+++	+++

Table 9 : Result of Phytochemical Screening of methanol extract of *Garcinia cowa* stem

4.2 DPPH Test of methanol extract of *Garcinia cowa* stem

Concentration	Absorbance of sample	Absorbance of ascorbic acid	% of inhibition of sample	% of inhibition of ascorbic acid
0	0	0	0	0
12.5	0.686	0.098	20.232	70.663
25	0.38	0.089	55.813	73.252
50	0.157	0.064	81.744	80.749
100	0.141	0.039	83.604	88.188
200	0.133	0.024	84.534	92.710

Table 10 : Result of absorbance and %of inhibition of methanol extract of *Garcinia cowa* stem and ascorbic acid

4.2.1Preparation of DPPH Scavenging Activity Curve:

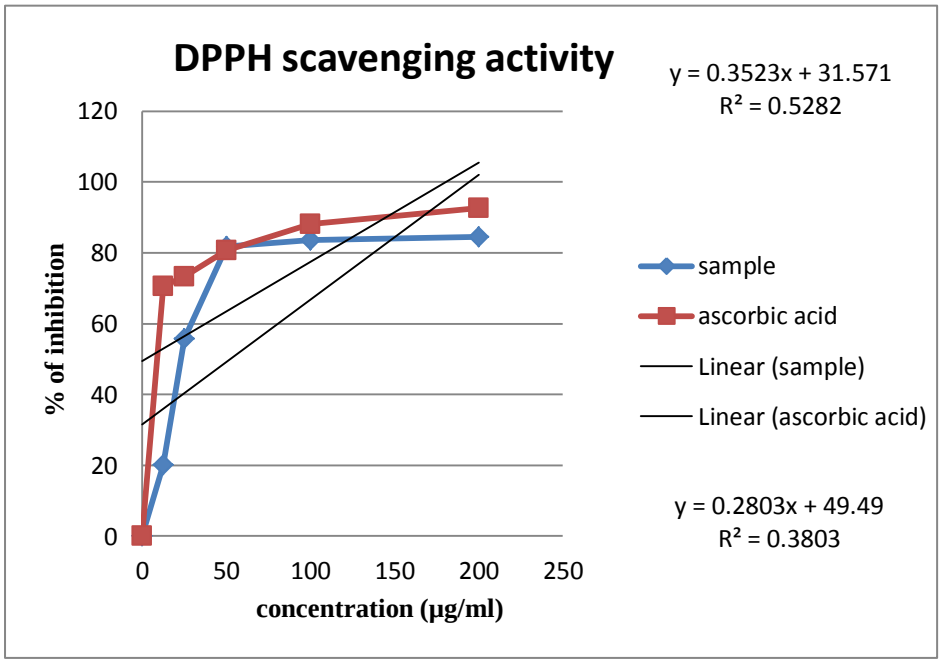


Figure 9: DPPH scavenging activity curve of methanol extract of *Garcinia cowa* stem

4.2.2 Results of DPPH Test of methanol extract of *Garcinia cowa* stem

Methanol extract of *G. cowa* stem /Ascorbic acid	**Regression Line**	**R^2 Line**	**IC_{50} Value (µg/ml)**
Methanol extract of *G. cowa* stem	Y=0.352x+31.57	R^2=0.528	52.358
Ascorbic acid	Y=0.280x+49.49	R^2=0.380	1.821

Table 11 : Result of DPPH test of methanol extract of *Garcinia cowa* stem

4.3 Total Phenol content of methanol extract of *Garcinia cowa* stem

4.3.1 Preparation of Standard Curve for Gallic Acid:

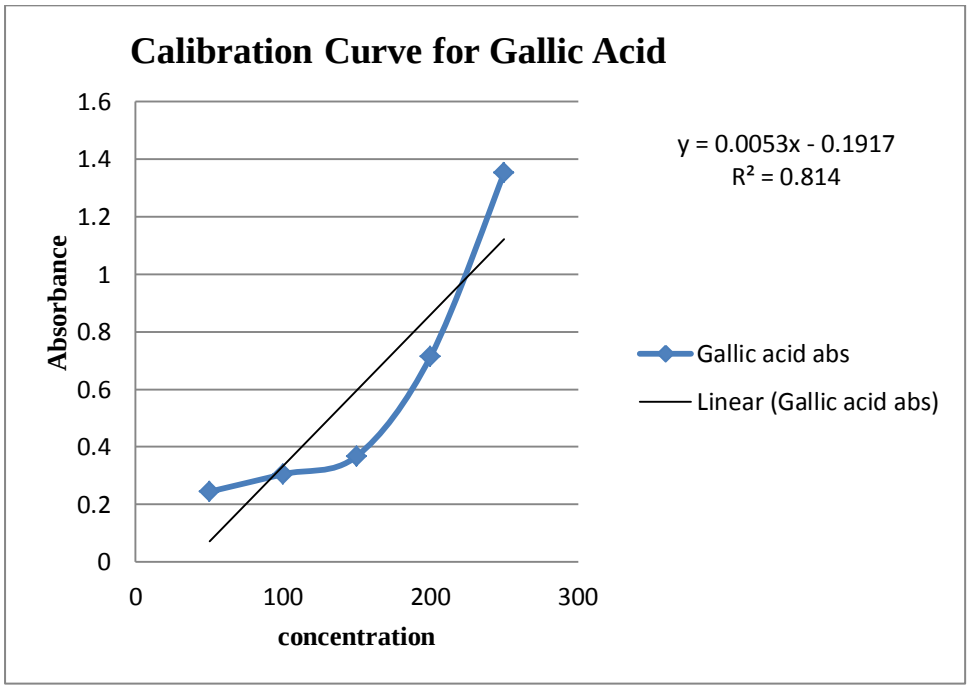


Figure 10: Standard Curve of Gallic Acid

4.3.2 Results of Total Phenol content:

Serial no	Absorbance of methanol extract of *G. cowa* stem	Standard equation	Value	Mean	Standard Deviation	Total Phenol content (mg/g Gallic Acid equivalent)
1	0.618		161.8			
2	0.529	Y=0.005x-0.191	144	149.6	10.577	149.6±10.577
3	0.524		143			

Table 12 : Result of Total Phenol content of methanol extract of *Garcinia cowa* stem

4.4 Antimicrobial screening of methanol extract of *Garcinia cowa* stem

Name of microorganism	Zone of inhibition		
	MeOH extract of G. cowa stem (400µg/disc)	MeOH extract of G. cowa stem (800µg/disc)	Kanamycin (30µg/disc)
Bacillus sereus	-	-	25mm
Bacillus megaterium	-	9mm	25mm
Bacillus subtilis	7mm	8mm	28mm
Salmonella paratyphi	-	-	40mm
Salmonella typhi	7mm	11mm	26mm
Vibrio parahemolyticus	8mm	8mm	26mm
Staphylococcus aureus	-	-	25mm
E.Coli	7mm	8mm	35mm
Shigella dysenteriae	8mm	9mm	25mm
Pseudomonas aureus	7mm	8mm	30mm

Table 13 : Result of zone of inhibition of methanol extract of *G. cowa* and Kanamycin

Discussion

Garcinia cowa has been used as a medicinal plant for the treatment of various diseases like dysentery, Stomach ache, Cramps, Headache. It is effective as an astringent, used in spasm. Many reports are available on the antiviral, antibacterial, antifungal, and anti-inflammatory, antimalarial properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. Due to its huge therapeutic use I get interested to do experiment on this plant.

Plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation methanol extract of *Garcinia cowa* stem of the family Clusiaceae used in various disease conditions. In my experiment it shows very positive result for antioxidant and antimicrobial activity.

Phytochemical screening showed that the methanol extract of *Garcinia cowa* stem was rich in phytochemical constituents. Such as- Glycoside, Flavonoid, Steroid, Carbohydrates and Tannin compounds. Thus further research is needed to work out the active medicinal compounds present in this extract; used for the treatment of various types of diseases.

In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect. The antioxidant activity was measured by DPPH scavenging assay and total Phenol content tests. The IC_{50} values of DPPH test was 52.358µg/ml for methanol extract of *Garcinia cowa* stem. The Total Phenol content was 149.6±10.57mg/g equivalent to Gallic Acid for Methanol extract of *Garcinia cowa* stem. The result are express as mean ± standard deviation where the n=3. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. It becomes difficult to describe the all properties selectively to any one group of constituents without further studies, which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active principles present in these plants.

The Antimicrobial Activity of the methanol extract of *Garcinia cowa* stem was tested against ten microorganisms. The highest antimicrobial activity was shown against *Vibrio parahemolyticus* and *Shigella dysenteriae.* The diameter of zone of inhibition was 8 mm(400µg/disc) compared to the 26 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc.It showed moderate activity against *Bacillus subtilis and E.Coli* (7mm). In case of 800µg/disc, the highest zone of inhibition of *Garcinia cowa* was 11mm for *Salmonella typhi* where the standard Kanamycin zone of inhibition was 26 mm. It also showed good activity against *Bacillus megaterium* (9mm) *and Pseudomonas aureus* (8mm).It showed no activity against *Bacillus sereus, Salmonella paratyphi* and *Staphylococcus aureus.* So, the methanol extract of the *Garcinia cowa* stem showed good antimicrobial activity against the selected microorganisms and thus further studies must be conducted to isolate the pure compounds and to evaluate their antimicrobial activity by using more advanced methods.

Conclusion

From the result of my study, it can be concluded that, using in vitro experiments established that methanol extract of *Garcinia cowa* stem inhibits the bacterial growth. In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect. For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. In my experiment it shows very positive result for anti-oxidant activity, antimicrobial activity. The antimicrobial activity of the plant extracts were tested against ten potentially bacterial pathogenic by using disc diffusion method at different concentrations of the methanol extracts of *Garcinia cowa* stem to understand the most effective activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

References:

Academic library (2014). Biochemical assays for Antioxidant activity assessment. Available:http://academlib.com/17949/environment/biochemical_assays_antioxidant_activity_assessment#664

Academic library (2014). Biotransformation of Waste Biomass into High Value Biochemicals. Available: http://academlib.com/17945/environment/antioxidants.

Balunas, M. and Kinghorn, A. (2005). Drug discovery from medicinal plants. Life Sciences, [online] 78(5), pp.431-441. Available at:

http://www.sciencedirect.com/science/article/pii/S0024320505008799 [Accessed 20 May. 2017]

Bhabani, S. J., Guddadarangavvanahally, K. J. and Kunnumpurath, K.S. (2002). Organic Acids from Leaves, Fruits, and Rinds of *Garcinia cowa*. Journal of Agricultural and Food Chemistry, 50 (12), pp.3431–3434

Chuah, L. O., Yeap, S. K., Ho, W. Y., Beh, B. K. and Alitheen, N. B. (2012). In Vitro and In Vivo Toxicity of Garcinia or Hydroxycitric Acid: A Review. Available at: https://www.hindawi.com/journals/ecam/2012/197920/.[Last accessed 22th May,2017]

Cheek, M. (2004). "*Garcinia kola*". IUCN Red List of Threatened Species. Version 2008. International Union for Conservation of Nature. [Last accessed 20th May,2017]

Hassan,B.A.R. (2012). Medicinal Plants (Importance and Uses). Available at: https://www.omicsonline.org/medicinal-plants-importance-and-uses-2153-2435.1000e139.php?aid=10654.

Hassler, M.. (2015). Taxonomy. Available at: http://zipcodezoo.com/index.php/Garcinia_cowa.[Last accessed 20th may, 2017]

Husni, E., Nahari, F., Wirasti, Y. and Wahyuni, F.S.(2015) . Cytotoxicity study of ethanol extract of the stem bark of asam kandis (Garcinia cowa Roxb.) on T47D breast cancer cell line. Asian Pacific Journal of Tropical Biomedicine, 5(3), pp.249-252.

Kuete,V.,Komguem,J.,Beng,V.P.,Meli,A.L.,Tangmouo,J.G.,Etoa,F.X. and Lonsi,D. (2007). Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* Oliver (Clusiaceae). Available at: http://www.sciencedirect.com/science/article/pii/S0254629907000051.[Last accessed 22th May,2017]

Li, L. (2000).Traditional Chinese medicine. Available at:

https://www.ncbi.nlm.nih.gov/books/NBK326625/.[Last accessed 18th may,2017]

Morgenstern, k. (2003). Plants and People. Available at:

http://www.sacredearth.com/ethnobotany/plantsnpeople.php. [Last accessed 20th may 2017]

Martinez and Staba. (2005). Importance of Medicinal Plants. Available at :

https://ayuvedaherbs.wordpress.com/.[Last accessed 20 May 2017]

Morgan, K. (2002).Medicine of the Gods: Basic Principles of Ayurvedic Medicine [http://www. compulink.co.uk/~mandrake/ayurveda.htm]

Mpbd.info, (2014). Medicinal Plants of Bangladesh. [Online] Available at:

http://www.mpbd.info/ [Accessed 20May2017]

Negi, P. S., Jayaprakasha, G. K. and Jena, B. S. (2010). Evaluation of Antioxidant and Antimutagenic Activities of the Extracts from the Fruit Rinds of *Garcinia cowa.* International Journal of Food Properties. 13 (6), pp.1256-1265.

Na, Z., Song, Q. and Hu, H. (2013). A New Prenylated Xanthone from Latex of *Garcinia cowa* Roxb. Records of Natural Products. 7 (3), pp.220-224.

Petrovska,B.(2012).Historical Review of Medicinal plants' Usage. Pharmacognosy Reviews,6(11),pp.1-5.

Pillai, D. S., Prabhasankar, P., Jena, B. S. and Anandharamakrishnan, C. (2012). Microencapsulation of *Garcinia Cowa* Fruit Extract and effect of its use on Pasta Process and Quality. International Journal of Food Properties,15(3), pp.590-604.

Panthong, k., Towatana, N. H. and Panthong, A.(2009). Cowaxanthone F, a new tetra oxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. Available at: https://www.researchgate.net/publication/237152677.Cowaxanthone F, a new tetra oxygenated xanthone and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. [Last accessed 20th may, 2017]

Panthong, K., Pongcharoen, W., Phongpaichit, S. and Taylor, W.C. (2006). Tetra oxygenated xanthones from the fruits of *Garcinia cowa*. Phytochemistry, *67*(10), pp.999-1004.

Ritthiwigrom, T., Laphookhieo, S. and Pyne,S.G. (2013). Chemical constituents and biological activities of *Garcinia cowa* Roxb. Available at: http://ro.uow.edu.au/cgi/viewcontent.cgi?article=2084&context=smhpapers.[Last accessed 18th may,2017].

Shaw, D.(1998).History of Use of Traditional Herbal Medicines. Available at: https://www.ncbi.nlm.nih.gov/books/NBK326625/.[Last accessed 18th may,2017]

Schulz, V., Hänsel, R. & Tyler, V.E.. (2001).The role of herbal medicines in traditional healing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK326625/. [Last accessed 18th may,2017]

Saito, H.. (2000). Japanese traditional medicine. Available at: https://www.ncbi.nlm.nih.gov/books/NBK326625/. [Last accessed 18th may,2017]

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

Shen,J.,Tian,Z.and Yang,J,S. (2007). The Constituents from the Stems of *Garcinia cowa* Roxb. and Their Cytotoxic Activities. Available at: https://www.researchgate.net/publication/6122281.The Constituents from the Stems of *Garcinia cowa* Roxb and Their Cytotoxic Activities. [Last accessed 21may,2017].

Tyler, V.E. . (2000). Introduction of traditional herbal medicines into Europe, the USA and other developed countries. Available at: https://www.ncbi.nlm.nih.gov/books/NBK326625/.[Last accessed 18th may,2017]

Uddin,S.B.(2014).*Garcinia cowa Roxb.* Available at: http://www.mpbd.info/plants/garcinia-cowa.php.[Last accessed 20th may, 2017]

U.S. National Library of Medicine (2017). Antioxidants. Available at: https://medlineplus.gov/antioxidants.html

Xu, G., Kan, W. L. T., Zhou, Y. and Xu, H. X. (2010). Cytotoxic Acylphloroglucinol Derivatives from the Twigs of *Garcinia cowa*. Journal of Natural Products 73 (2), pp.104-108.

Xia, Z., Zhang, H., Xu, D. and Xu, H.(2015). Xanthones from the Leaves of *Garcinia cowa* Induce Cell Cycle Arrest, Apoptosis, and Autophagy in Cancer Cells. Available: https://www.researchgate.net/publication/279194750.Xanthones from the Leaves of *Garcinia cowa* Induce Cell Cycle Arrest Apoptosis and Autophagy in Cancer Cells.[Last accessed 20th may, 2017].

Yusuf, M., Wahab, M., Yousuf, M., Chowdhury, J. and Begum, J. (2007). Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. Bangladesh Journal of Plant Taxonomy, [online] 14(2), pp.15-28.

Available at: http://www.scribd.com/doc/4003274/Some-Common-Medicinal-Plants-of Bangladesh-With-scientific-name-and-use [Accessed 20May. 2017]

Yap, L. V.,Noor, N. M., Clyde, M. M. and Chin, H. F.(2011). Cryopreservation of *Garcinia cowa* shoot tips by vitrification: The effects of sucrose preculture and loading treatment on ultrastructural changes in meristematic cells. Available at: https://www.researchgate.net/publication/51499332.Cryopreservation of *Garcinia cowa* shoot tips by vitrification.The effects of sucrose preculture and loading treatment on ultrastructural changes in me.[Last accessed 22th May,2017].

Zahid,H.(2016).Introduction and Importance of Medicinal plant and Herbs. Available : https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and herbs_mtl. [Last accessed 20th may, 2017]