Antioxidant and Anti-microbial Activity Assessment of Methanol Extract of *Garcinia cowa* Leaves

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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Department of Pharmacy

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Declaration

I, **Farhena Afrose Tanha** hereby declare that this dissertation, entitled '**Antioxidant and Antimicrobial Assesment of Methanol Extract of *Garcinia cowa* Leaves** ' 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* leaves**' is a research work carried out by Farhena Afrose (ID: 2013-1-70-038) in 2017, under the supervision and guidance of me, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled is a research work carried out '**Antioxidant and Antimicrobial Investigations Of Methanol Extract Of *Garcinia cowa* stem**' by **Farhena Afrose Tanha** (ID: 2013-1-70-038), 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 strength.

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Abstract:

Bangladeshi local plant *Garcinia cowa* (cow tree) is an abundant source of bioactive phytochemicals. Phytochemical investigations of the plant parts indicated that the leaves 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 seen to posess different pharmacological activities. *Garcinia cowa* was screened for their anti-microbial and anti-oxidant activities . The part which was being evaluated for its activity was methanol extract of leaves. Methanol extracts of *Garcinia cowa* leaves exhibited strong anti-oxidant and antibacterial activities . First phyto-chemical test was carried out where methanol extract of leaves showed high content of flavonoid & tannins. DPPH scavenging activity test & total phenolic content test is rendered to monitor the inhibitory activity against oxidation. In DPPH scavenging activity test the IC_{50} or inhibitory concentration of the sample was obtained (28.30 µg/ml) much more higher than the standard (Ascorbic acid; IC_{50} was 1.8194) compound. This shows that the methanol extract of leaves have strong anti-oxidant property. Methanol extract of leaves also showed high total phenoilic content (223.1333 mg/g) which indicates that leaf extract of this particular solvent (MeoH) has strong anti-oxidant property .Anti-microbial activity test was also carried out using methanol extract of leaves which showed different sized zone of inhibition for different strains of bacteria . The sensitive bacteria to this extract were *Bacillus sereus, Bacillus megaterium , Salmonella typhi , Shigella dysenteriae .Pseudomonas aureaus* where highest millimeters of zone of inhibition was formed.

Keywords: Antioxidant activity, Anti-microbial activity,Phyto-chemical test,DPPH scavenging activity,Total phenolic content test, Anti-microbial test ,IC_{50}, Zone of inhibition.

CHAPTER-1

Introduction

1.1 Medicinal plants：

Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defence against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex.

The earliest historical records of herbs are found from the Sumerian civilisation, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines, while Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*, forming the basis of pharmacopoeias for some 1500 years. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols, and terpenes.

Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. The annual global export value of pharmaceutical plants in 2012 was over US$2.2 billion. (Lichterman, B. L. ,2004)

Curry Plant

Badam or Indian Almond Tree

Chikun Tree

Shami

Biswul Plant

1.2 History:

1.2.1 Prehistoric times:

Plants, including many now used as culinary herbs and spices, have been used as medicines from prehistoric times. Spices have been used partly to counter food spoilage bacteria, especially in hot climates, and especially in meat dishes which spoil more readily. Angiosperms (flowering plants) were the original source of most plant medicines. Human settlements are often surrounded by weeds useful as medicines, such as nettle, dandelion and chickweed. Humans were not alone in using herbs as medicines: some animals such as non-human primates, monarch butterflies and sheep ingest medicinal plants to treat illness. Plant samples from prehistoric burial sites are among the lines of evidence that Paleolithic peoples had knowledge of herbal medicine. For instance, a 60 000-year-old Neanderthal burial site, "Shanidar IV", in northern Iraq has yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies, A mushroom was

found in the personal effects of *Ötzi the Iceman*, whose body was frozen in the Ötztal Alps for more than 5,000 years. The mushroom was probably used to treat whipworm.

1.2.2 Ancient times :

In ancient Sumeria, hundreds of medicinal plants including myrrh and opium are listed on clay tablets. The ancient Egyptian Ebers Papyrus lists over 800 plant medicines such as aloe, cannabis, castor bean, garlic, juniper, and mandrake.

Fig 1.2.2.1:Ancient Ebers Papyrus

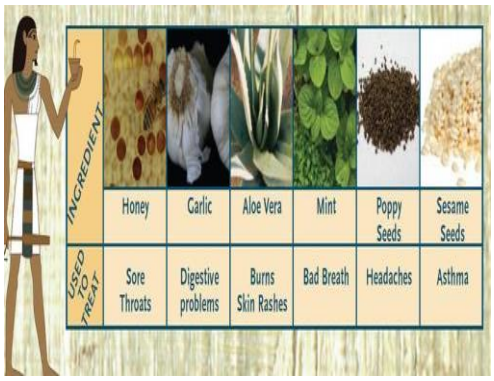


Fig 1.2.2.3 :Medicinal Plant list of Ebers Papyrus

From ancient times to the present, Ayurvedic medicine as documented in the Atharva Veda, the Rig Veda and the Sushruta Samhita has used hundreds of pharmacologically active herbs and spices such as turmeric, which contains curcumin. The Chinese pharmacopoeia, the *Shennong Ben Cao Jing* records plant medicines such as chaulmoogra for leprosy, ephedra, and hemp. This was expanded in the Tang Dynasty *Yaoxing Lun*. In the fourth century BC, Aristotle's pupil Theophrastus wrote the first systematic botany text, *Historia plantarum*. In the first century AD, the Greek physician Pedanius Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*; it remained the authoritative reference on herbalism for over 1500 years, into the seventeenth century. (Collins, Minta 2000).

1.2.2.4 Aloe-Vera

1.2.2.5 Cannabis

1.2.2.6 Poppy plant

1.2.3 Middle Ages:

In the Early Middle Ages, Benedictine monasteries preserved medical knowledge in Europe, translating and copying classical texts and maintaining herb gardens. Hildegard of Bingen wrote *Causae et Curae* ("Causes and Cures") on medicine. In the Islamic Golden Age, scholars translated many classical Greek texts including Dioscorides into Arabic, adding their own commentaries.

1.2.3.1 "*Causae et Curae* " by Hildegard of Bingen

Herbalism flourished in the Islamic world, particularly in Baghdad and in Al-Andalus. Among many works on medicinal plants, Abulcasis (936–1013) of Cordoba wrote *The Book of Simples*, and Ibn al-Baitar (1197–1248) recorded hundreds of medicinal herbs such as *Aconitum*, nux vomica, and tamarind in his *Corpus of Simples*.

1.2.3.4 *Aconitum*

1.2.3.5 *Nux vomica*

1.2.3.6 Tamarind Tree

Avicenna included many plants in his 1025 *The Canon of Medicine*. Abu-Rayhan Biruni, Ibn Zuhr, Peter of Spain, and John of St Amand wrote further pharmacopoeias.(Tapsell, L. C. 2006)

1.2.4 Early Modern :

The Early Modern period saw the flourishing of illustrated herbals across Europe, starting with the 1526 *Grete Herball*. John Gerard wrote his famous *The Herball or General History of Plants* in 1597, based on Rembert Dodoens, and Nicholas Culpeper published his *The English Physician Enlarged*. Many new plant medicines arrived in Europe as products

of Early Modern exploration and the resulting Columbian Exchange, in which livestock, crops and technologies were transferred between the Old World and the Americas in the 15th and 16th centuries. Medicinal herbs arriving in the Americas included garlic, ginger, and turmeric; coffee, tobacco and coca travelled in the other direction. In Mexico, the sixteenth century *Badianus Manuscript* described medicinal plants available in Central America (Billing, Jennifer,1998)

1.2.4.1 Garlic plant

1.2.4.2 Ginger plant

1.2.4.3 Coffee Plant

1.2.4.4 Turmeric Plant

1.3 Phyto-chemistry:

1.3.1 Alkaloids:

Alkaloids are bitter-tasting chemicals, very widespread in nature, and often toxic. There are several classes with different modes of action as drugs, both recreational and pharmaceutical. Medicines of different classes include atropine, scopolamine, and hyoscyamine (all from nightshade), berberine (from plants such as *Berberis* and *Mahonia*). Picture of some alkaloids is given below :

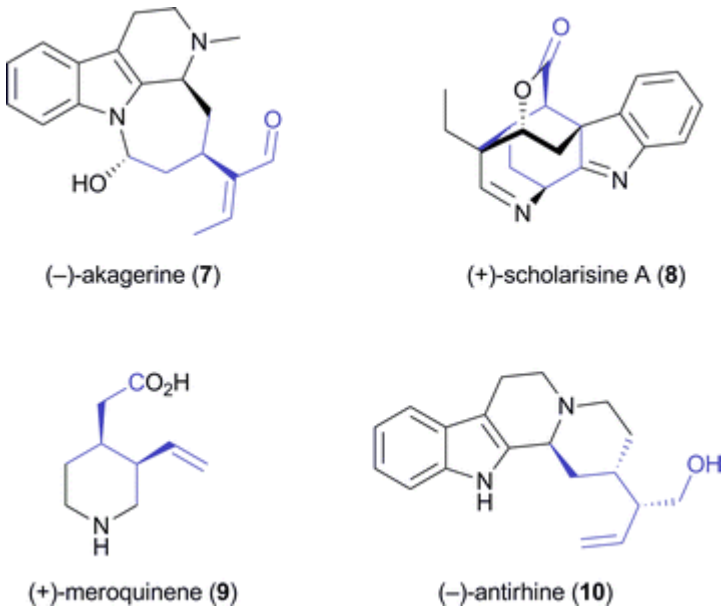
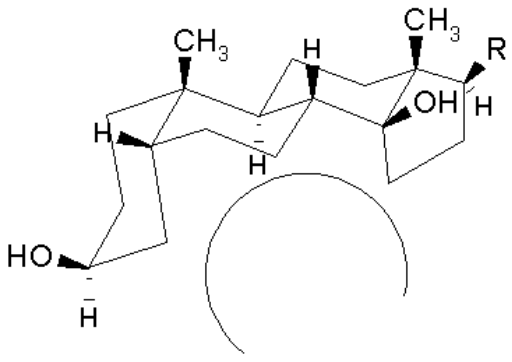


Fig 1.3.1.1 : Different types of alkaloids

1.3.2 Glycosides:

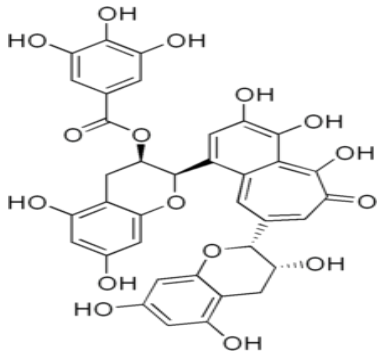
Anthraquinone glycosides are found in the laxatives senna, rhubarb,and *Aloe*. The cardiac glycosides are powerful drugs from plants including foxglove and lily of the valley. They include digoxin and digitoxin which support the beating of the heart, and act as diuretics.



1.3.2.1 Cardiac Glycoside

1.3.3 Polyphenols :

Polyphenols of several classes are widespread in plants. They include the colourful anthocyanins, hormone-mimicking phytoestrogens, and astringent tannins. In Ayurveda, the astringent rind of the pomegranate is used as a medicine, while polyphenol extracts from plant materials such as

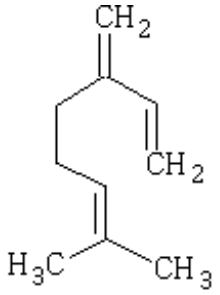


1.3.3.1 Polyphenol

grape seeds are sold for their potential health benefits despite the lack of evidence. Plants containing phytoestrogens have been used for centuries to treat gynaecological disorders such as fertility, menstrual, and menopausal problems. Among these plants are *Pueraria mirifica*, kudzu, angelica, fennel, and anise.

1.3.4 Terpenes :

Terpenes and terpenoids of many kinds are found in resinous plants such as the conifers. They are strongly aromatic and serve to repel herbivores. Their scent makes them useful in essential oils .



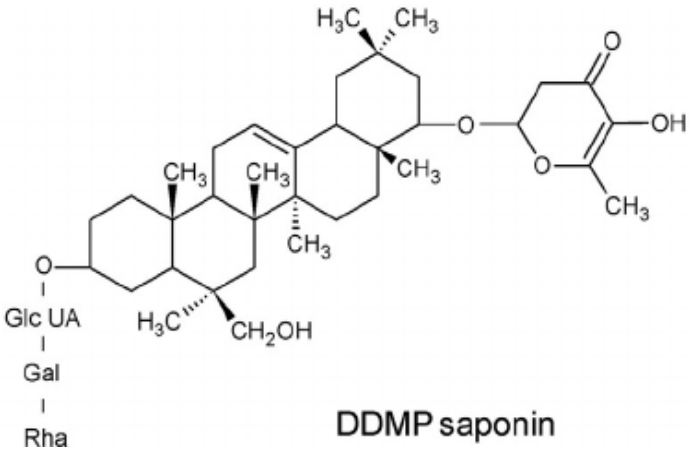
7-methyl-3-methyleneocta-1,6-diene
Myrcene

1.3.5 Saponins :

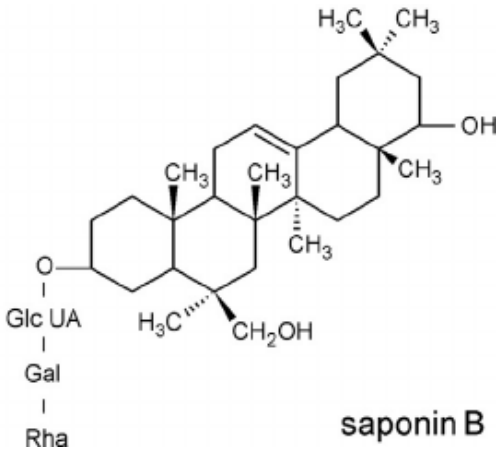
Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. One research use of the saponin class of natural products involves their complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection. In addition, the amphipathic nature of the class gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes.

Saponins from the *Gypsophila paniculata* (baby's breath) plant have been shown to significantly augment the cytotoxicity of immunotoxins and other targeted toxins directed against human cancer cells. The research groups of Professor Hendrik Fuchs (Charité University, Berlin, Germany) and Dr David Flavell (Southampton General Hospital, United Kingdom) are working together toward the development of *Gypsophila* saponins for use in combination with immunotoxins or other targeted toxins for patients with leukaemia, lymphoma and other cancers.Saponins have also been used as adjuvants in vaccines, e.g. Quil A component QS-21, isolated from the bark of *Quillaja saponaria* Molina, to stimulate both the Th1 immune response and the production of cytotoxic T-lymphocytes (CTLs) against exogenous antigens. This makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens as well as for therapeutic cancer vaccines but with the aforementioned side-effects of hemolysis.In their

use as adjuvants in the production of vaccines, toxicity associated with sterol complexation remains a major issue for attention.



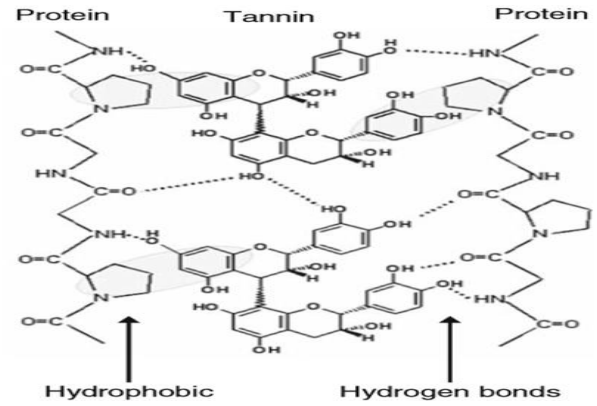
DDMP saponin



saponin B

1.3.6 Tannins :

A tannin (or tannoid) is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times. (Sumner, Judith ,2000)



1.3.6.1 Tannins

1.4 Cultivation:

Medicinal plants demand intensive management. Different species each require their own distinct conditions of cultivation. The World Health Organization recommends the use of rotation to minimise problems with pests and plant diseases. Cultivation may be traditional or may make use of conservation agriculture practices to maintain organic matter in the soil and to conserve water, for example with no-till farming systems. In many medicinal and aromatic plants, plant characteristics vary widely with soil type and cropping strategy, so care is required to obtain satisfactory yields. (Carrubba, 2012)

1.5 Uses:

Plant medicines are ubiquitous in pre-industrial societies, while some 7,000 conventional medicines such as aspirin, digitalis, opium, and quinine derive directly from traditional plant medicines, accounting for around a quarter of the modern pharmacopoeia. They are in general far cheaper, and many can be home-grown or picked for free. Further, pharmaceutical companies have made use of the herbal knowledge of indigenous peoples around the world to search for new drug candidates. In India, where Ayurveda has been practised for centuries, herbal remedies are the responsibility of a government department, AYUSH, under the Ministry of Health & Family Welfare. Traditional Chinese medicine makes use of a wide variety of plants, among other materials and techniques.

Complementary and alternative medicines including herbal therapy are widely used in the Western world, for example by over a third of Americans.

Plant medicines including opiates, cocaine and cannabis have both medical and recreational uses. Different countries have at various times made some uses of drugs illegal, partly on the basis of the risks involved in taking psychoactive drugs. (*Kala,*2007)

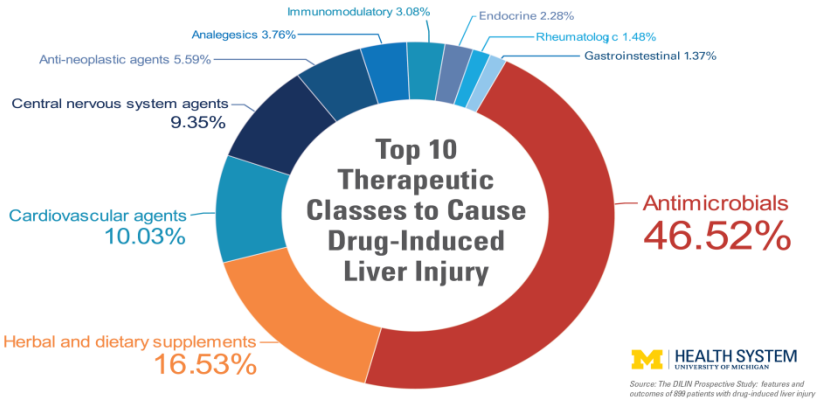
1.4.1 3mg supplement of **Herbal Melatonin** capsule

1.6 Effectiveness:

Plant medicines have often not been tested systematically, but have come into use informally over the centuries. By 2007, clinical trials had demonstrated potentially useful activity in nearly 16% of herbal medicines; there was limited in vitro or in vivo evidence for roughly half the medicines; there was only phytochemical evidence for around 20%; 0.5% were allergenic or toxic; and some 12% had effectively never been studied scientifically. According to Cancer Research UK, "there is currently no strong evidence from studies in people that herbal remedies can treat, prevent or cure cancer". Modern knowledge of medicinal plants is being systematised in the Medicinal Plant Transcriptomics Database, which provides a sequence reference for the transcriptome of some thirty species. Hundreds of compounds have been identified using ethnobotany, investigating plants used by indigenous peoples. There is renewed interest in the discovery of therapeutically useful substances from medicinal plants.(Cravotto G, 2010)

1.7 Safety :

Plant medicines can cause adverse effects and even death, whether by side-effects of their active substances, by adulteration or contamination, by overdose, or by inappropriate prescription. Many such effects are known, while others remain to be explored scientifically. There is no reason to presume that because a product comes from nature it must be safe: the existence of powerful natural poisons like atropine and nicotine shows this to be untrue. Further, the



high standards applied to conventional medicines do not always apply to plant medicines, and dose can vary widely depending on the growth conditions of plants: older plants may be much more toxic than young ones, for instance. Pharmacologically active plant extracts can interact with conventional drugs, both because they may provide an increased dose of similar compounds, and because some phytochemicals interfere with the body's systems that metabolise drugs in the liver including the cytochrome P450 system, making the drugs last longer in the body and have a more powerful cumulative effect. Plant medicines can be dangerous during pregnancy. Since plants may contain many different substances, plant extracts may have complex effects on the human body. (Nekvindová, J ,2007)

1.9 Quality:

Herbal medicines and supplements are not always tested for purity, and there is some concern about adulteration and inclusion of allergens such as soy and wheat in some products. (O'Connor ,2013)

1.10 Medicinal Plants in Bangladesh :

Chemical constituents and medicinal uses of 449 medicinal plants growing and available in Bangladesh are listed. Information on their habitats, cultivation, economic importance and pharmacological actions is provided. Genera include: *Abroma, Abutilon, Acanthus, Achyranthes, Adenanthera, Adiantum, Alangium, Allamanda, Ammannia, Amorphophallus, Ampelygonum, Anagallis, Anamirta, Andrographis, Anisomeles, Anthocephalus, Aphanamixis, Aquillaria, Ardisia, Argemone, Aristolochia, Artabotrys, Asclepias, Bacopa, Baliospermum, Barleria, Barringtonia, Basella, Belamcanda, Benincasa, Boerhavia, Borassus, Borreria, Boswellia, Butea, Callicarpa, Calophyllum, Calotropis, Calycopteris, Cannabis, Canscora, Capparis, Cardiospermum, Careya, Carissa, Carthamus, Cassytha, Catharanthus, Celosia, Centella, Cissus, Clausena, Cleome, Clerodendrum, Clitoria, Coccinia, Cocculus, Coleus, Costus, Crataeva, Crinum, Croton, Cryptolepis, Cullen, Curculigo, Cycas, Dactyloctenium, Dendrophthae, Digera, Dillenia, Drypetes, Ecbolium, Eclipta, Elaeocarpus, Elephantopus, Embelia, Emilia, Enhydra, Entada, Equisetum, Euryale, Excoecaria, Feronia, Flacourtia, Gardenia, Garuga, Glinus, Gloriosa, Glycosmis, Gnaphalium, Grangea, Gynandropsis, Garcinia, Hedyotis, Helicteres, Heliotropium, Hemidesmus, Hiptage, and Holarrhena*. Pharmacopoeial plant drugs of Bangladesh are tabulated.(Ghani, A.,1998)

1.11Traditional use of Medicinal plants in Bangladesh :

The rural population of Bangladesh has traditionally depended on folk medicinal healers for treatment of their ailments. These healers use medicinal plants as their primary source of medicinal formulations. Rural patients are more dependent on traditional or folk medicinal healers for treatment of urinary tract infections (UTIs) and sexually transmitted diseases (STDs) for a number of reasons including lack of access to modern medical facilities, clinging to traditional approaches, and finally hesitancy to relate this form of illnesses in front of unknown doctors. Since the traditional healer usually resides in the same village or in an adjoining area, the patient is more comfortable in seeking them for treatment. We

conducted an ethnomedicinal survey among the traditional healers of various ethnic groups and in several regions of the country to obtain information on medicinal plants used to treat UTIs and STDs. Interviews were conducted in the local dialect or language about plant parts used, ailments treated, formulations, and dosages. Thirty-one species were reported by traditional healers as being used for UTIs, including leucorrhea, frequent or infrequent urination, cloudy urination and burning sensations during urination. Ten species were reported to be used against STDs like syphilis and gonorrhea. (Hossan, S, 2010).

Folk medicinal practitioners (Kavirajes) of Bangladesh are consulted for treatment of various ailments by a substantial segment of the rural and urban population of the country. The major element that distinguishes the folk medicinal practitioners from other forms of medical practices is their use of simple formulations of medicinal plants for treatment. The plant(s) used by the Kavirajes for treatment of any specific ailment vary considerably in the various parts of the country, and such differences exist even among Kavirajes of adjoining villages. The objective of the present study was to conduct an ethnomedicinal survey among the Kavirajes of two villages, namely Babla and Terbaria, which lies in Tangail district in the central portion of the country. Each village had one practicing Kaviraj. Leaves constituted the major plant part used, being used 48.7% of the time. From the number of plants used, it appeared that gastrointestinal tract disorders formed the major complaint of the patients with 5 plants used for treatment of various complaints like constipation, diarrhea, indigestion, and loss of appetite. Four plants each were used for treatment of pain, and skin disorders (scabies, eczema), and as blood purifier. Four plants were used for treatment of diseases in cattle. Among other ailments treated by the Kavirajes were tuberculosis, sexual disorders, urinary problems, infections, fever, hepatic disorders, kidney problems, pneumonia, stomach stones, diabetes, swellings, debility, helminthiasis, hypertension, vitamin C deficiency, tumor, and poisoning. One plant was used to maintain the body in good health and so served as a preventive measure instead of a curative effect. Since a number of allopathic medicines have been derived from medicinal plants, the plants reported in the present survey can, following scientific inquiry, form novel sources of newer drugs.

The predominantly rural population of this district is served by traditional medicinal practitioners (Kavirajes), who utilize medicinal plants for treatment of various ailments. Since the medicinal plants used by the Kavirajes can vary from region to region depending

on the availability of plant species and the background training of the Kavirajes, it was the objective of the present study to conduct an ethnomedicinal survey among the Kavirajes of Dinajpur district, Bangladesh to gather information on the medicinal plants used by the Kavirajes of this district. A secondary objective of the present survey was to determine which medicinal plants can also serve as functional foods and can be taken on a regular basis for general well-being as well as treatment for ailments. A number of plants were found that could serve this dual purpose. The plants included Amomum subulatum, Bixa orellana, Cajanus cajan, Carissa carandas, Cinnamomum tamala, Cinnamomum zeylanicum, Coccinia grandis, Dillenia indica, Ferula asafoetida, Manilkara zapota, Mentha arvensis, Moringa oleifera, Nymphaea nouchali, Phyllanthus emblica, Spilanthes acmella, Syzygium aromaticum, Terminalia belerica, and Terminalia chebula. Functional foods can be important sources of macro- and micro-nutrients and at the same time used for prevention or cure of diseases. As such, the above plants can play important roles in the maintenance of body health, particularly of the poorer sections of the population.

Sylhet division lies in the north-eastern corner of Bangladesh and comprises of four districts--Sylhet, Habiganj, Sunamganj, and Moulvibazar. The division contains a diversity of floral species, some of which are quite distinct from the rest of the country. A randomized ethnomedicinal survey was conducted among the folk medicinal practitioners of Komolganj in Moulvibazar district, Gulapganj of Sylhet district, and Chunarughat of Habiganj district. Informed consent was obtained from the healers and the survey was conducted with the help of a semi-structured questionnaire. In the present survey, the methodology employed was that of the guided field-walk, where the healers took the interviewers to localities from where they collected their medicinal plants and pointed out the plants besides describing the plant parts used and the ailments that they were used for. Plant specimens were collected from the field, dried in situ and identification completed at the Bangladesh National Herbarium. Information on 107 plant species distributed into 53 families was obtained. The Asteraceae family contributed the largest number of plant species (seven) followed by the Euphorbiaceae, Fabaceae and Rutaceae families (six each). Leaves comprised the major plant part used for the treatment of different ailments (48.3%) followed by fruit (15.9%) and bark (10.3%). Most plants were used to treat common ailments like gastrointestinal disorders, helminthiasis, debility, pain, skin problems, respiratory problems, fever, bleeding

from cuts and wounds, urinary tract problems and sexual disorders. However, a number of plants were also used to treat more complicated ailments like cardiovascular disorders, hepatic disorders, epilepsy and cancer or tumors. In the majority of cases, a single plant part was used for treatment of any given ailment. Folk medicine in Bangladesh has a history of usage going back thousands of years. The medicinal plants used by the folk medicinal healers thus possess considerable potential for discovery of lead compounds or novel compounds that may serve as the source of effective modern drugs.

The Santals form the largest tribal community in northern Bangladesh reside primarily in Rajshahi and Rangpur Divisions, where they live in the districts of Rajshahi, Rangpur, Thakurgaon, Dinajpur, and Panchagarh. Although they are fast losing their traditional medicinal practices, they still have their own medicinal practitioners who rely mostly on medicinal plants for treatment of a variety of ailments. The traditional medicinal practices vary quite extensively between the twelve clans of the Santals. The objective of the present study was to conduct an ethnomedicinal survey amongst the Soren clan of the Santal community residing in two villages of Tanor Santal Para in Rajshahi district to collect information on their use of medicinal plants. Plant specimens as pointed out by the practitioners were collected and pressed on the field and identification completed at the Bangladesh National Herbarium. Information on 53 medicinal plants distributed into 32 families was obtained in this survey. Ailments treated by these plants included skin disorders, respiratory tract disorders, gastro-intestinal disorders, sexual dysfunctions, sexually transmitted diseases, diabetes, helminthiasis, pain, urinary problems, filariasis, leprosy, tuberculosis, epilepsy, snake bite, enlarged heart, and paralysis. The medicinal plants

used by the Santals merit further scientific studies for some of their formulations are used to treat diseases like diabetes, paralysis, enlarged heart, tuberculosis, and filariasis for which modern medicine has no known cure or medicines have developed resistant vectors.

In Mymensingh district of Bangladesh it was found out that medicinal plants that they use for treatment of diabetes. It was found that the tribal practitioners of the Marakh sect of the Garos use twelve medicinal plants for treatment of diabetes. These plants were Lannea coromandelica, Alstonia scholaris, Catharanthus roseus, Enhydra fluctuans, Terminalia chebula, Coccinia grandis, Momordica charantia, Cuscuta reflexa, Phyllanthus emblica,

Syzygium aqueum, Drynaria quercifolia, and Clerodendrum viscosum. A review of the scientific literature demonstrated that almost all the plants used by the Garo tribal practitioners have reported antidiabetic and/or antioxidant properties and have enormous potential for possible development of new and efficacious antidiabetic drugs.(*M Rahmatullah ,2012)*

1.12 Plants under the *Garcinia* Class:

Garcinia is a plant genus of the family Clusiaceae native to Asia, Australia, tropical and southern Africa, and Polynesia. The number of species is highly disputed, with various sources recognizing between 50 and about 300. Commonly, the plants in this genus are called saptrees, mangosteens (which may also refer specifically to the purple mangosteen, *G. mangostana*), garcinias or, ambiguously, "monkey fruit".Many species are threatened by habitat destruction, and at least *G. cadelliana* from South Andaman Island is almost or even completely extinct already. The fruits are a food source for several animals, such as the archduke butterflies (*Lexias*) of tropical eastern Asia which relish the sap of overripe mangosteens. *Garcinia* species are evergreen trees and shrubs, dioecious and in several cases apomictic. The fruit is a berry with fleshy endocarp, which in several species is delicious. Some selected species are :

- *Garcinia acutifolia*
- *Garcinia afzelii*
- *Garcinia aristata*
- *Garcinia atroviridis – asam gelugur* (Indonesian), *asam gelugor* (Malaysian), *asam keping* (Malaysian)
- *Garcinia benthami*
- *Garcinia bifasciculata*
- *Garcinia brassii*
- *Garcinia brevipedicellata*
- *Garcinia burkillii*
- *Garcinia cadelliana*

- *Garcinia cambogia*
- *Garcinia cantleyana*
- *Garcinia cerasifer* (H.Perrier) P.F.Stevens
- *Garcinia clusiaefolia*
- *Garcinia costata*
- *Garcinia cymosa* (K.Schum.) I.M.Turner & P.F.Stevens
- *Garcinia decussata*
- *Garcinia diversifolia*
- *Garcinia dulcis – mundu, rata*
- *Garcinia echinocarpa*
- *Garcinia elliptica*
- *Garcinia epunctata*
- *Garcinia eugeniaefolia*
- *Garcinia forbesii*
- *Garcinia fragraeoides*
- *Garcinia gardneriana – bacupari*

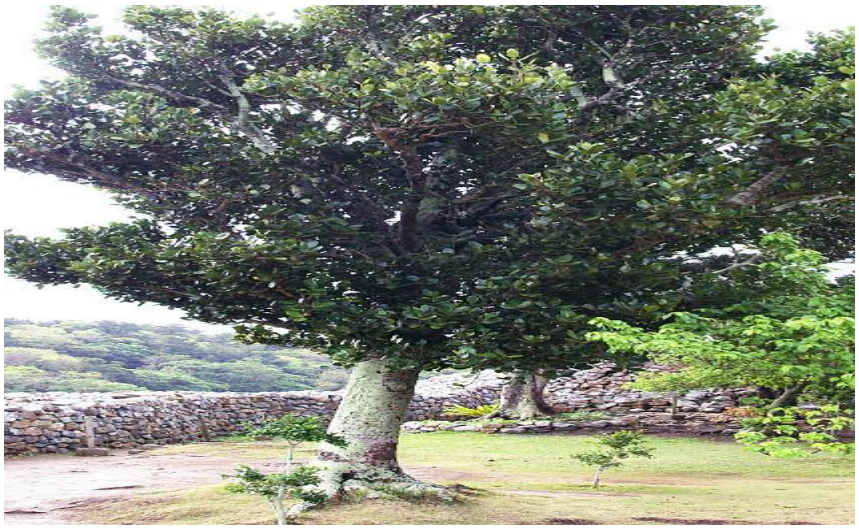
Fig1.12.1 : Habitat of Garcinia Class Plants Around the World

- *Garcinia gerrardii* Harv. ex Sim
- *Garcinia gummi-gutta* – gambooge, garcinia cambogia (a former scientific name now used as a common name), brindleberry, brindall berry, Malabar tamarind, goraka (sinhala, Sri Lanka), *goraka, Uppaage* ಉಪ್ಪಾಗೆ *in Kannada,*

  kodumpulli (Kerala), *kudampuli* (Tamil)
- *Garcinia hanburyi* – Hanbury's garcinia
- *Garcinia hendersoniana*
- *Garcinia hermonii*
- *Garcinia hessii* – lemon saptree
- *Garcinia heterandra*
- *Garcinia holttumii*
- *Garcinia hombroniana* – seashore mangosteen, *pokok bruas* (Malay)

- *Garcinia hopii* H.Toyama & V.S.Dang, 2017 – Bidoup Nui Ba National Park, southern Vietnam
- *Garcinia humilis* – achachairú, achacha
- *Garcinia imberti*
- *Garcinia indica* – wild mangosteen, *amsol, bhinda, biran, katambi, kokum, punarpuli (ಪುನರ್ಪುಳಿ) in Kannada kodam-puli, ratamba*, etc.
- *Garcinia intermedia* – lemon drop mangosteen, *charichuelo*
- *Garcinia kingii Garcinia kola* – bitter kola
- *Garcinia linii*
- *Garcinia livingstonei* – African mangosteen, Lowveld mangosteen, Livingstone's garcinia, *imbe*
- *Garcinia loureiroi* - (Khmer: ស្វាយជុំ), sour fruit and leaves widely used in Cambodian cuisine, specifically in a version of the dish *samlar machu*[6]
- *Garcinia madruno* (Humb. & Bonpl. ex Kunth) Hammel – lemon drop mangosteen, *ungüento maría, tierra amarillo,* madroño
- *Garcinia maingayi*
- *Garcinia mannii*
- *Garcinia mangostana* – purple mangosteen
- *Garcinia mestonii*
- *Garcinia minutiflora*
- *Garcinia monantha*
- *Garcinia montana*
- *Garcinia morella – batuan* (Hiligaynon), *ireevalsinni* (Tamil)
- *Garcinia multiflora* Champ. – *hạt điều màu* (Vietnamese)
- *Garcinia murtonii*
- *Garcinia nigrolineata* Planch. ex T.Anderson
- *Garcinia oliveri*
- *Garcinia opaca*
- *Garcinia paucinervis*

- *Garcinia pedunculata*
- *Garcinia portoricensis*
- *Garcinia prainiana* – button mangosteen, *cherapu*
- *Garcinia pseudoguttifera* Seem. – *mo'onia* (Tongan)
- *Garcinia pushpangadaniana*
- *Garcinia pyrifera*
- *Garcinia quaesita*
- *Garcinia rubro-echinata*
- *Garcinia schomburgkiana*
- *Garcinia scortechinii*
- *Garcinia semseii*
- *Garcinia sessilis* Seem. – *heilala* (Tongan), *seilala* (Samoan)
- *Garcinia staudtii*
- *Garcinia subelliptica* Merr. – *fukugi* (Japanese)
- *Garcinia terpnophylla*
- *Garcinia thwaitesii*
- *Garcinia tinctoria*
- *Garcinia travancorica*
- *Garcinia uniflora*
- *Garcinia vitiensis*
- *Garcinia warrenii* F.Muell.
- *Garcinia wightii*
- *Garcinia xanthochymus* – *asam kandis* (Indonesian)
- *Garcinia zeylanica* (Cheek, M. 2004)



1.12.2 ***Garcinia cowa*** tree in Bangladesh

1.13 *Garcinia cowa* Tree Description:

Family: Clusiaceae

Synonyms: Garcinia kydia

Other names: Bhava, chenhek,cow fol.

Cowa is a lesser known edible fruit found in the states of East India (Assam, Mizoram, Bengal, Bihar and Orissa). It is also found in the Andaman and Nicobar Islands. It occurs wild frequently in evergreen and semi evergreen forests or along streams in deep valleys. Besides India, it is also reported to grow in South China, Bangladesh, Malaysia, Laos, Cambodia and Vietnam. In Mizoram, cow is cultivated as a subsidiary crop in Citrus, banana and arecanut orchards. Trees 8-12 m tall, 15-20 cm in diam; bark dark brown; branches many, borne toward top of trunk, horizontal but usually distally pendulous, slender; twigs dark brown, striate.

Cowa foliage

Petiole 0.8-1.5(-2) cm; leaf blade lanceolate or oblong-lanceolate, 6-14 × 2-5 cm, papery, midvein raised abaxially, impressed adaxially; secondary veins 12-18 pairs, near margin joining together; tertiary veins conspicuous on both surfaces, base cuneate, sometimes slightly decurrent, margin cartilaginous, involute, apex acuminate or long acuminate, rarely acute or obtuse.

Dioecious; male flowers 3-8, terminal or axillary, in an umbel; umbel shortly

Flowers of cowa

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

stigma radiately 4-8-lobed, papillate, 6-7 mm high. Fruit opaquely yellow-brown, ovoid-globose, oblique, 5-6 × 4-5 cm in diam., 4-8-sulcate, usually apiculate, pinkish red, looking similar to tomato. Seeds 2-4, narrow, fusiform, slightly curved, ca. 2.5 cm, rough.

Fruits of cowa

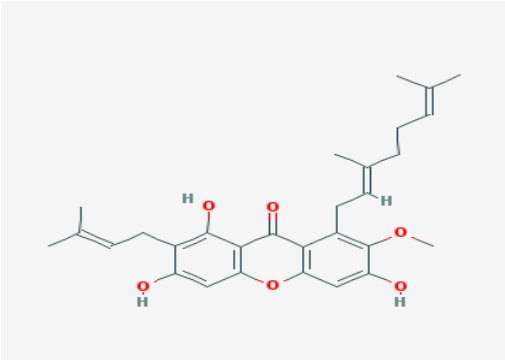
In East India, the sun dried slices of this fruit are used to treat dysentery. The young leaves are cooked and eaten as a vegetable. The bark is used for dying clothes yellow.Cowa tree also produces a yellow gum resin which resembles gamboge. New trees are raised from seed. These are planted at a distance of 8 m from each other the bearing starts in 4-5 years. (Dr. Chiranjit Parmer,2008)

1.14 Chemical compounds found in *Garcinia Cowa* :

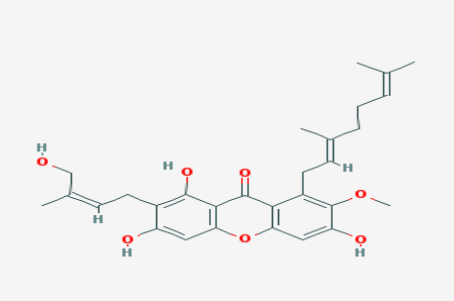
1.14.1 Xanthones :

Generally xanthones from the bark of *Garcinia cowa*, namely 7-*O*-methylgarcinone E , cowanin , cowanol , cowaxanthone , and β-mangostin are found. 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 [20]; 7- O-methylgarcinone E (36), 1,5,6-trihydroxy-3-methoxy-4-(3-

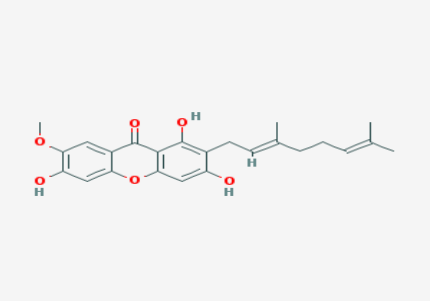
hydroxyl-3-methylbutyl)xanthone (59), 4-(1,1-dimethyl-prop-2-enyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthen-9(9H)-one (61) and 1,5-dihydroxy-3methoxy-6′,6′-dimethyl-2H-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone (62) from the stem [18, 33]; and cowaxanthone F (55) from the twig [16]. Most of these xanthones showed interesting biological activities. (Likhitwitayawuid, 1998)



1.14.1.1Cowanin

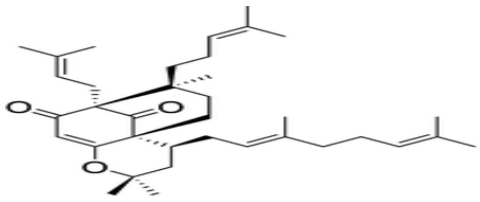


1.14.1.2 Cowanol

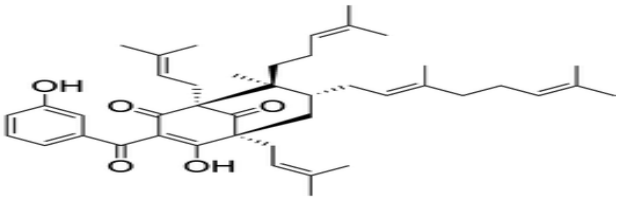


1.14.1.3 Cowaxanthone

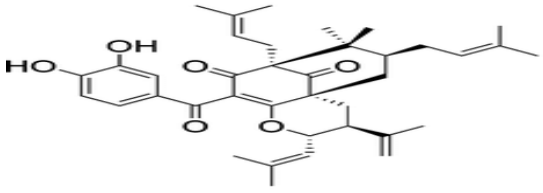
An unusual polyprenylated acylphloroglucinol derivative unsubstituted at C-2 and C-6, garcicowin together with three other new garcicowins B−D, and nine known analogues, was isolated and characterized from the twigs of *Garcinia cowa*. (Xu, G.,2010)



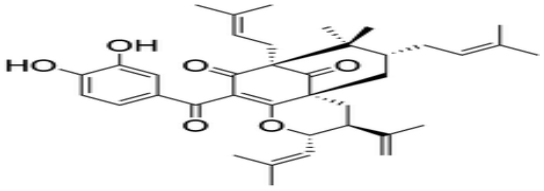
Garcicowin A (1)



Garcicowin B (2)

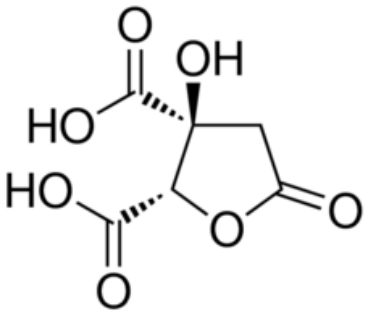


Garcicowin C (3)

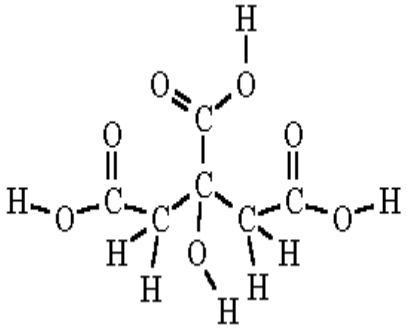


Garcicowin D (4)

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 respectively. Seventyeight of these compounds have been identified from the plant and several have interesting pharmacological activities.(Ritthiwigrom, T,2013) . Organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa* (*G. cowa*) were determined. The major organic acid was found to be (−)-hydroxycitric acid present in leaves, fruits, and rinds to the extent of 1.7, 2.3, and 12.7%, respectively. (−)-Hydroxycitric acid lactone, and oxalic and citric acids are present in leaves, fruits, and rinds in minor quantities. (Jena, B.S 2002)



Hydroxycitric acid lactone



Citric Acid

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

1.14.2 Depsidone

Depsidones comprise benzoic acid and phenol skeletons condensed at the ortho-positions through ester and ether linkages. This class of natural products is well known in the Garcinia species . However, cowadepsidone was the first and only known depsidone from G. cowa. It was isolated from the twig extract.

1.14.3 Flavonoids

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

1.14.4 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 (compounds 14-27 in Table 1) 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) [7, 26, 27], 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 .

1.14.5 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), β-sitosterol (30) and

stigmasterol (31) [24]. None of these compounds were further studied for their biological activities. However, these compounds which were isolated from other plants had been investigated for their biological activity. Activities.From the root bark of Terminalia avicennioides exhibited antibacterial activity against Bacillus Calmette-Guerin (BCG) with an MIC of 4.9 μg/mL [38]. Friedelin (28) and stigmasterol (31) isolated from the leaf of Jatropha tanjorensis were tested against human pathogenic microorganisms, i.e. Gram-positive bacteria: Bacillus cereus, B. subtilis, S. aureus and S. epidermis; Gram-negative bacteria: Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, P. vulgaris, Salmonella paratyphi, S. paratyphi A, Vibrio alcaligenes and V. cholera; and fungi: Aspergillus fumigatus, Candida albicans, Microsporum gypseum and Trichophyton rubrum using the agar-well diffusion and disk diffusion methods [39]. Friedelin (28), at the concentration of 2 μg/mL, showed maximum activity with 37-40, 17-40 and 31-33 mm of clear zone diameter against these three types of microorganisms respectively [39], while stigmasterol (31) at the same concentration exhibited maximum activity with 13-15, 8-17 and 7-8 mm of clear zone diameter respectively [39]. Daucosterol (29) from the roots of Astragalus membranaceus had no growth-inhibitory effect by direct contact but possessed immunomodulatory effect against disseminated candidiasis caused by Candida albicans [40]. β-Sitosterol (30) and stigmasterol (31), isolated from the bark of Grewia tiliaefolia, at the same concentration of 1 μg/mL showed antibacterial activity against the Gram-negative bacterium P. aeruginosa (ATCC-20852) with 18 and 20 mm of clear zones respectively and against Klebsiella pneumonia (MTCC-618) with 15 and 15 mm of clear zones respectively as determined by the agar diffusion method .

1.15 Use of *Garcinia* Class:

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

intermedia) with yellow fruit that look like a wrinkled lemon, and the thin-skinned orange button mangosteen (*G. prainiana*).In addition, mangosteen rind (exocarp) extract is used as a spice. It figures prominently in Kodava culture, and *G. multiflora* is used to flavour and colour the famous *bún riêu* soup of Vietnam, where this plant is known as *hạt điều màu*. *Garcinia gummi-gutta* yields a spice widely used in South Asia, in particular in Kerala, where it is called *kodumpulli*.Most species in *Garcinia* are known for their gum resin, brownish-yellow from xanthonoids such as mangostin, and used as purgative or cathartic, but most frequently – at least in former times – as a pigment. The colour term gamboge refers to this pigment. Extracts of the exocarp of certain species – typically *G. gummi-gutta*, but also purple mangosteen – are often contained in appetite suppressants such as Hydroxycut, Leptoprin or XanGo. But their effectiveness at normal consumption levels is unproven, while at least one case of severe acidosis caused by long-term consumption of such products has been documented. Furthermore, they may contain significant amounts of hydroxycitric acid, which is somewhat toxic and might even destroy the testicles after prolonged use. Bitter kola (*G. kola*) seeds are used in folk medicine. *G. mannii* is popular as a chew stick in western Africa, freshening the breath and cleaning the teeth.*G.subelliptica*,called *fukugi* in Japanese,isthe floralemblem of Mobuto and Tarama on Okinawa. The Malaysian town of Beruas – often spelled "Bruas" – derives its name from the seashore mangosteen (*G. hombroniana*), known locally as *pokok bruas*.(WONG, L.P,2008)

1.16 Traditional Uses of *Garcinia Cowa* in Bangladesh:

- 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 hydroxycitric acid (HCA) might increase how long untrained women are able to exercise. However, it does not seem benefit men in the same way.
- Used for Weight loss. Research on the effect of Garcinia on weight loss is inconsistent. Some research shows that taking Garcinia extract that contains 50% hydroxycitric acid (HCA) for 8-12 weeks doesn't decrease fat breakdown or energy expenditure in overweight people. However, other research suggests that it might improve weight loss when taken for 12 weeks. Taking a specific Garcinia product containing 60% HCA (Super CitriMax InterHealth Nutriceuticals) by mouth in three doses daily 30 to 60 minutes before meals for 8 weeks, together with a healthy diet, seems to improve weight loss more than just diet alone. But other research shows that adding this specific Garcinia product to cereal bars or tomato juice and consuming them before lunch and dinner for 2 weeks does not improve weight loss. Reasons for the inconsistent results might be the dose, duration of treatment, or formulation of *Garcinia* extract that was used (Sibly Aziz, M.A ,2007)

Garcinia is safe for most people when taken by mouth for 12 weeks or less. Long-term safety is unknown. Garcinia can cause nausea, digestive tract discomfort, and headache. Currently there is no information for interactions, The appropriate dose of garcinia depends on several factors such as the user's age, health, and several other conditions. At this time, there is not enough scientific information to determine an appropriate range of doses for garcinia. Natural products are not always necessarily safe and dosages can be important. So relevant directions on product labels should be followed and consultation with the pharmacist or physician or other healthcare professional before using is needed.(World Conservation Monitoring Centre,1998)

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CHAPTER-2

Literature Review

2.1 Antibacterial activity of the extracts from the Fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria :

The crude hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* were studied for their antibacterial activity against some foodborne pathogens and spoilage bacteria such as *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentrations (MICs) of the extracts determined by the agar dilution method were ranging from 15 to 500 µg/ml and 300 to 1250 µg/ml for *G. cowa* and *G. pedunculata*, respectively. However, the hexane and chloroform extracts from the fruit rinds of *G. cowa* exhibited marked inhibitory effect against all the test organisms and were more effective than that of *G. pedunculata* extracts. The antibacterial activity of all the extracts was more pronounced against the tested Gram-positive bacteria than the tested Gram-negative bacterium. Furthermore, this study is the first report on the *in vitro* antibacterial activity of extracts from the fruit rinds of *G. cowa* and *G. pedunculata*. (Negi, P.S ,2008)

2.2 Tetraoxygenated xanthones from the fruits of *Garcinia cowa:*

Tetraoxygenated xanthones, cowaxanthones A–E, together with 10 previously reported tetraoxygenated xanthones, were isolated from the crude hexane extract of the fruits of *Garcinia cowa*. Cowaxanthone 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. Tetraoxygenated xanthones: cowaxanthones 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 tetraoxygenated xanthones (cowaxanthones A–E: 1, 2, 3, 4 and 5), together with 10 known tetraoxygenated xanthones: l,6-dihydroxy-

3,7-dimethoxy-2-(3-methyl-2- butenyl)xanthone (6), fuscaxanthone C (7), 7-O-methylgarcinone E (8), b-mangostin (9), cowanol (10), mangostanin (11), 6-O-methylmangostanin (12), cowanin (13a-mangostin (14) cowaxanthone (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, K.,2006)

2.3 Cytotoxic Acylphloroglucinol Derivatives from the Twigs of *Garcinia cowa :*

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 cells. (Xu, G ,2010)

2.4 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) and1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl) xanthone (2), have been isolated together with six known xanthones: 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone (3), dulxanthone A (4), 1,5,6-trihydroxy-3,7-dimethoxyxanthone(5),1,7-dihydroxyxanthone(6),1,3,5-trihydroxy-6-

methoxyxanthone(7),1,3,6,7-tetrahydroxyxanthone (8), from the stems of *Garcinia cowa* (Guttiferae).(Shen, J. 2006)

2.5 7-*O*-methylgarcinone e from *Garcinia cowa:*

The stem bark of *Garcinia cowa* furnished a new xanthone which was characterized as 7-*O*-methyl-garcinone E. (Likhitwitayawuid, K.,1997)

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

The dormancy breaking and storage behavior of Garcinia cowa Roxb. seeds were investigated. The seeds of G. cowa had 8–11 months dormancy in their natural habitat. Seeds were matured and dispersed at the end of the rainy season (mid-late August to late September) and were scatter-hoarded by rodents as food for winter after the seeds had fallen to the ground. Seedlings often emerged in the forest during the rainy season (May to August) the following year. Intact seeds of G. cowa failed to germinate after being sown at 30 °C for 120 d and the mean germination time (MGT) of seeds cultured in a shade (50% sunlight) nursery was 252 d. The most effective method of breaking dormancy was to remove the seed coat totally, which reduced the MGT to 13 d at 30 °C. Germination was also promoted by partial removal of the seed coat (excising the hilum and exposing the radicle) and chemical scarification (immersion in 1% H2O2 for 1 d). (LIU, Y,2005)

2.7 Antiaflatoxigenic and antioxidant activities of *Garcinia* extracts :

The effect of hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* on the growth and aflatoxin production in *Aspergillus flavus* was studied using peanut powder as a model food system. The growth of *A. flavus* was

completely inhibited by the hexane and chloroform extracts from *G. cowa* and chloroform extract from *G. pedunculata* at 3000 ppm concentration, which was considered as the minimum inhibitory concentration (MIC). The MIC for the hexane extract of *G. pedunculata* was at 4000 ppm. Both the extracts from *G. cowa* inhibited aflatoxin B_1production upto 100% at a lower concentration of 2000 ppm. It was observed that, at lower concentration of the extracts from *G. cowa* and *G. pedunculata*, the degree of inhibition of aflatoxin production was much higher than the inhibition of fungal growth. The hexane and chloroform extracts from *G. cowa* and *G. pedunculata* were also studied for their antioxidant capacity by the formation of phosphomolybdenum complex at 100 ppm concentration and reducing power by potassium ferricyanide reduction method at various concentrations. Hexane and chloroform extracts from *G. cowa* showed higher antioxidant capacity than *G. pedunculata* extracts. Similarly, both the extracts from *G. cowa* showed higher reducing power than the extracts from *G. pedunculata*. The antiaflatoxigenic activities of the extracts from *G. cowa* and *G. pedunculata* may be due to their effective antioxidative properties, which could suppress the biosynthesis of aflatoxin.(Joseph, G.S ,2005)

2.8 Microencapsulation of *Garcinia Cowa* Fruit Extract and Effect of its use on Pasta Process and Quality:

Microencapsulation is employed to protect bioactive ingredients in foods and is also used for their controlled release at targeted sites. Hydroxycitric acid ((-)-HCA) is present in the fruits of certain species of *Garcinia* and it has been studied extensively for its unique regulatory effect on fatty acid synthesis, lipogenesis, appetite, and weight loss. Since hydroxycitric acid is hygroscopic in nature, it is very difficult to convert liquid extract from the fruits of *Garcinia* into dried powder. Hence, microencapsulation of *Garcinia cowa* fruit extract was performed in a pilot-scale co-current spray dryer with whey protein isolate as a wall material. In this study, two different wall-to-core ratios (1:1 and 1.5:1) and dryer outlet temperatures (90 and 105°C) were used for assessing the encapsulation efficiency. The results in this study showed that the microencapsulation efficiency (based on HPLC analysis) and antioxidant properties (based on 2,2-diphenyl-1-picrylhydrazyl assay) were higher at 90°C outlet temperature of the spray dryer using 1.5:1 wall-to-core ratio feed. Further, the

spray-dried powders were incorporated into pasta processing and evaluated its quality characteristics. The results of this study demonstrated that incorporation of powder spray-dried at 90°C outlet temperature with 1.5:1 wall-to-core pasta exhibited higher antioxidant activity as well as better cooking and sensory characteristics. (Pillai, D.S ,2012)

2.9 Antibacterial dihydrobenzopyran and xanthone derivatives from *Garcinia cowa* stem barks :

Two new compounds, garciniacowol (1) and garciniacowone (2) along with 15 known compounds were isolated from the stem barks of *Garcinia cowa*. Their structures were determined by intensive spectroscopic methods. The structure of 1 was a symmetrical dimeric dihydrobenzopyran derivative, whereas the framework of 2 was a triprenyl caged-xanthone precursor. The antibacterial activities against *Escherichia coli* TISTR 780, *Salmonella typhimurium* TISTR 292, *Staphylococcus aureus* TISTR 1466, and methicillin-resistant *S. aureus* (MRSA) SK1 of the isolated compounds were also evaluated. Compounds 2 and 9 exhibited good antibacterial activity against MRSA SK1 with the same minimum inhibitory concentration (MIC) value of 2 μg/mL. Moreover, compound 2 also showed good antibacterial activity against *S. aureus* with an MIC value of 2 μg/mL. (Siridechakorn, I,2012)

2.10 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 hydroxyanisole (BHA) showed 91.7, 93.7, and 98.0% antioxidant activity, respectively, whereas, at 50 ppm

concentration the radical scavenging activity was 83.3, 86.3, and 88.5%, respectively, through DPPH method. At a concentration of 5000 μg/plate, hexane extract exhibited strong antimutagenicity against the mutagenicity of sodium azide in both the tester strains of Salmonella typhimurium (TA-100 and TA-1535). Chloroform extract showed strong antimutagenicity in both the tester strains at a concentration of 2500 μg/plate and above. However, the chloroform extract exhibited higher antioxidant and antimutagenic activities than that of hexane extract. This study showed that both the extracts from the fruit rinds of *G. cowa* possess antioxidant and antimutagenic properties

2.11 Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from Garcinia cowa :

A new tetraoxygenated xanthone, cowaxanthone F (1), as well as four known compounds, morelloflavone (2), volkensiflavone (3), morelloflavone-7″-*O*-glucoside (fukugiside, 4), and 1,6-dihydroxyxanthone (5), were isolated from the crude acetone extract of the twigs of Garcinia cowa (Guttiferae). All compounds (1–5) were tested for antioxidant activity against DPPH (diphenylpicrylhydrazyl), hydroxyl, and superoxide radicals; only morelloflavone (2) and morelloflavone-7″-*O*-glucoside (4) exhibited high potency. Eight tetraoxygenated xanthones from the fruits of G. cowa, cowaxanthones A–D (6–9), cowanin (15), α-mangostin (16), mangostanin (17), and cowanol (18), were also investigated for anti-inflammatory activity using ethyl phenylpropiolate (EPP)-induced ear edema. Assessment at 30, 60, and 120 min revealed that cowaxanthones B–D (7–9), cowanin (15), and α-mangostin (16) exhibited significant anti-inflammatory activity when compared to phenylbutazone, while cowaxanthone A (6), mangostanin (17), and cowanol (18) showed less activity. .(Negi, P.S,2010)

2.12 The constituents from the stems of *Garcinia cowa* and their cytotoxic activities :

An ethanolic extract of the stems of G. cowa was concentrated and partitioned further into petroleum ether, ethyl acetate, n-butanol, water soluble fractions. The ethyl acetate and n-butanol fractions were subjected to chromatographic purification to afford three new compounds, garccowaside A–C (1–3) along with three known compounds S-()-5,7,30 ,50 -tetrahydroxyflavanone (4) þ)-3,5,7,30 ,50 -pentahydroxyflavanone (5) et al. 1997)quercetin Compound 1 was obtained as a pale yellow powder, m.p. 146–147 C. Its molecular formula was elucidated as C23H24O12 by analysis of its HR-FAB-MS. The UV spectrum of 1 showed the characteristic absorptions of flavanone derivative at 204, 225, 283 nm, and its IR spectrum contained some absorption bands due to an ester carbon (1732 cm1) and a g-pyrone (1641 cm1).

Compound 2 was also obtained as a pale yellow powder. The molecular formula was deduced as C25H18O12. The UV and IR spectrum absorptions were similar to 1, which suggested 2 to be a flavanone derivative. In the 1 H NMR spectrum, a singlet for three protons was observed at d 3.61, which indicated the presence of a methoxyl group substituted on a saccharide group. Seven phenolic compounds including five xanthones showed moderate cytotoxicity on three cancerous cell lines tested . Among them, dulxanthone A was the best one. Compounds 1–3 exhibited almost no remarkable growth inhibitory effect within the tested concentrations, which the aglycone owned some cytotoxic activities. Only compound 11 was different with other 1,3,5,6 oxygenation patterns of xanthones, which had no activities in three tests. (Shen, J.,2007)

2.13 Chemical constituents and biological activities of *Garcinia cowa* Roxb :

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 15-30 μg/mL)

but not E. coli (IC50s 250-500 μg/mL). The extracts were also found to inhibit the growth of Aspergillus flavus, a common fungal food contaminant which produces aflatoxin B1. The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (ca 40-60% at the same concentration) [22]. The methanol extracts of the leaves and twigs of G. cowa were evaluated for their ability to inhibit low-density lipoprotein peroxidation induced by copper ions. The twig extract had an IC50 value of 20.5 μg/mL and was more potent (higher % inhibition at 1000 μg/mL) than the leaf extract (IC50 not measured). The twig extract was more potent than the leaf extract on platelet aggregation of human whole blood induced by arachidonic acid, adenosine diphosphate and collagen. These activities may be due to the total phenolic content of these extracts, which were 19 and 61 mg of gallic acid equivalent per g of extract for the leaf and twig extracts respectively.

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 .

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 .

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.

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 IC50 values ranging between 3.87-8.58 µg/mL, and moderately inhibited KB and MCF-7 cancer cell lines with IC50 values ranging between 6.43-15.43 and 10.59- 21.38 µg/mL respectively [17]. Dulxanthone A (60) was found to be cytotoxic against the HepG2 cell line.

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-methyl-5β-(3,7,11,15- tetramethyl-hexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one (68) [32]. None of the isolated compounds from this class were tested for their biological activities.(Ritthiwigrom, T.,2013)

2.14 Seed dispersal, seed predation, and seedling spatial pattern of *Garcinia cowa* :

Seed dispersal is a multi-step process, which may include fruit removal, seed dissemination, post-dispersal seed predation, potential secondary dispersal, seed germination, and seedling establishment. Previous studies often focused on only one or a few of these stages. In this study, the complexity of the seed dispersal process of Garcinia cowa, a common climax undercanopy tree in seasonal rain forests of SW China is demonstrated.(Liu, Y.,2001)

Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone :

Twenty-two edible plant extracts were subjected to evaluation of their antibacterial activity against some gastrointestinal pathogenic bacteria, including Escherichia coli, Salmonella typhimurium, Salmonella typhi, Shigella sonnei and Helicobacter pylori using the disc diffusion and broth microdilution methods. Sixteen of the plant extracts exhibited antibacterial activity against one or more tested bacteria. Only Garcinia cowa leaf extracts exhibited antibacterial activity against all tested bacteria. Purification of the ethyl acetate extract of G. cowa leaves using an antimicrobial assay-guided isolation afforded a new polyprenylated benzophenone, chamuangone, that exhibited satisfactory antibacterial activity against Streptococcus pyogenes (minimum inhibitory concentration (MIC) 7.8 lg/ml), Streptococcus viridans and H. pylori (MICs 15.6 lg/ml), and Staphylococcus aureus, Bacillus subtilis and Enterococcus sp. (MICs 31.2 lg/ml). (Sakunpak, A.,2012)

2.15 Kaennacowanols A–C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa:*

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

2.16 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. The leaves of *G. cowa* were pulverized and extracted three times with acetone at room temperature. The acetone extract was suspended in hot water and partitioned with CH_2Cl_2. The CH_2Cl_2-soluble portion was subjected to repeated chromatography over silica gel, reversed-phase C_{18} silica gel, and preparative HPLC to afford 25 pure compounds . The CCK-8 assay was used to determine cell viability. Test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in culture medium for assays. Human cancer cell lines (HeLa, PANC-1, and A549) were cultured in RPMI 1640, DMEM or DMEM/F12 (1:1) medium containing 10% fetal bovine serum. Cells were maintained at 37 °C in a humidified environment under 5% CO_2. Cell proliferation assays were performed as previously described . Briefly, each cell line was seeded in a 96-well tissue culture plate at a predetermined density in 180 µL of complete medium, attached overnight and treated with test compound for 72 h. Then the medium was discarded and replaced with 10% CCK-8 in complete medium and the plates incubated for another 2 h. OD450 was measured with a SpectraMAX 190 spectrophotometer (MDS, Sunnyvale, CA, USA). Background absorbance was subtracted for all wells. Inhibition rate (IR) was determined. IR (%) = $(OD_{DMSO} - OD_{compound})/OD_{DMSO} \times 100\%$. Hypodiploid DNA and cell cycle arrest were evaluated as described previously. Briefly, after treatment of HeLa cancer cells with vehicle (0.1% DMSO) or test compound at the indicated concentrations and times, the cells were harvested by trypsinization and fixed with 70% (*v*/*v*) alcohol at 4 °C for 30 min. After washing with PBS, RNase (10 µg/mL) was added and incubated for 15 min at 37 °C to eliminate RNA interference. The cells were then treated with propidium iodide (PI) for another 30 min. The cells were washed, and the DNA content determined using FACSCalibur.

2.17 Cytotoxicity study of ethanol extract of the stem bark of asam kandis (*Garcinia cowa* Roxb.) on T47D breast cancer cell line :

The cytotoxicity of ethanol extract was carried out against human breast cancer cell line (T47D) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. The extract was added at various concentrations (0.1, 1, 10 and 100 μg/mL). The level of cytotoxicity was determined by calculating the level of IC_{50} that was based on the percentage of the cell death after 24 h treatment with the extract. Cell morphological changes were observed by using inverted microscope . The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that ethanol extract of *G. cowa* exhibited significant cytotoxic effect on T47D with IC_{50} value of (5.10±1.68) μg/mL. Morphological alteration of the cell lines after exposure to ethanol extract of *G. cowa* was observed under phase contrast microscope in a dose-dependent manner. The cytotoxicity effect of *G. cowa* on T47D was evaluated by MTT assay. The ethanol extract of *G. cowa* in multiple concentrations was used. The effective concentration was calculated from concentration-response curve. Based on the MTT assay, it was found that the ethanol extract of the stem bark of asam kandis had IC_{50} value of (5.10±1.68) μg/mL. The criteria of cytotoxicity for the crude extract, which was established by the U.S. National Cancer Institute, is IC_{50} <20 μg/mL in the preliminary assay .(Husni, E ,2015)

2.18 Evaluation of nutraceutical properties and antioxidant activity of *Garcinia cowa* Roxb. Ex Choisy fruits found in Assam (India) :

Fruits have been used as a dietary source of nutrition and play a vital role in improving our health. Various metabolic activities in our body results in the formation of the free radicals or reactive oxygen species (ROS) that leads to the onset of many diseases such as cancer, rheumatoid arthritis, liver diseases and atherosclerosis as well as in degenerative processes associated with ageing. Antioxidant compounds in diet play an important role as a health

protecting factor. The present study was aimed at investigating the phytochemical constituents and antioxidant activity of fruits obtaining from Garcinia cowa Roxb. ex choisy having ethno medicinal property. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical. The methanolic extract of mature fruits exhibited antioxidant activity significantly. The IC50 value of the methanolic extract was found to be 33.15±0.86 µg/ml. The study revealed that the fruits of this plant can be used as therapeutic agent as it would exert several beneficial effects by virtue of their antioxidant activity and also a rich source of nutrition in our diet system as it was rich in phytochemical constituents. (Sarma, A.,2015)

CHAPTER-3

Materials & Method

3.2Theory of Phytochemical Screening

3.2.1.1. 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.	

3.2.1.2 Test Compounds

Methanol extract of leaves of *Garcinia cowa.*

3.2.1.3 Preparation of Sample Solution

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

3.2.1.4 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. HClwas 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.

3.2.2Assessment of In Vitro Pharmacological Property

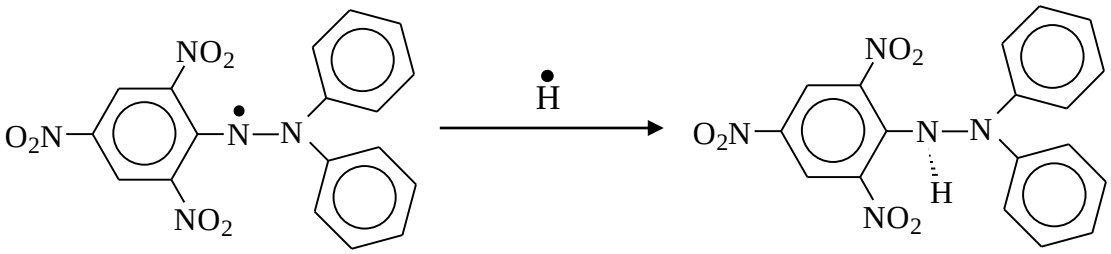
3.2.2.1 Determination of Antioxidant property

3.2.2.1.1DPPH 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.



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

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

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	Solution taken from stock	Solution taken	Adjust the volume by	Final

(µg/ml)	solution	from others	Absolute ethanol	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 (50µg/ml)	1 ml	2.0 ml
6.25	-	1 ml (25µg/ml)	1 ml	2.0 ml

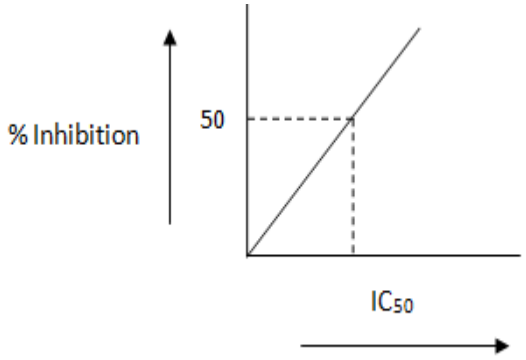
Procedure

- 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.



3.2.2.1.4 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 reductants and Mo(VI):

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

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

Preparation of 7.5% Sodium carbonate solution

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

Preparation of Standard solution

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

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

6.25	-	1 ml (12.5 µl/ml)	1000	2

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

Experimental Procedure

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

acid equivalents (GAE) was calculated using the following equation:

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

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

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

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

3.2.2.4 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).

3.2.2.4.1 Materials

Microorganisms

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

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:

Gram positiveBacteria	**Gram negativeBacteria**
Bacillus cereus *Bacillus subtilis* *Staphylococcus aureus*	*Escherichia coli* *Salmonella typhi* *Pseudomonas aeruginosa* *Serratiamarcescens* *Proteus mirrabillis*

Culture Media and Chemicals

- Nutrient agar media
- Ethanol
- Chloroform

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

Test Materials

The methanolic, chloroform and pet ether extract of *S. chelonoides*bark & leaves were tested against gram-positive and gram-negative bacteria.

3.2.2.4.2 Methods

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.

Sterilization Procedure:

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

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

Preparation of Subculture

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

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

Preparation of Discs

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.

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.

Preparation of sample discs with test samples

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

3.2.2.4.3 Placement of Disc and Incubation

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

3.2.2.4.4 Determination of Zone of Inhibition

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

Chapter -4

Result & Discussion

4.1 Result of Phyto-Chemical test *Garcinia cowa*: *Garcinia cowa* showed negative result in almost all the tests but showed distinctive positive result in flavonoid and steroid test.It has also high content of xanthine & fatty acid in its leaf.

Table 4.1.1 : Results of chemical group tests of the methanolic extract of *G.cowa* leaves :

Leaf extract of *G.cowa*	**Alkaloid**	**Terpinoids**	**Carbohydrates**	**Tanin**	**Flavonoid**	**Saponin**	**Steroid**
In Methanol	–	–	++	+++	+++	–	+

(+++): highly present,(++): moderately present,(+): slightly present,(-): absent

4.2 Result of DPPH Scavenging activity test :

Leaf methanol extract of *Garcinia cowa* showed significant amount of DPPH scavenging activity compared with ascorbic acid .The percent of inhibition of standard was monitored highest at the concentration of 200 µg/ml which was 92.7105.The percent of inhibition of leaf methanol was also highest at the concentration of 200 µg/ml which was 90.69767.The result shows that the leaf extract of methanol has high anti-oxidant or free radical inhibition property .

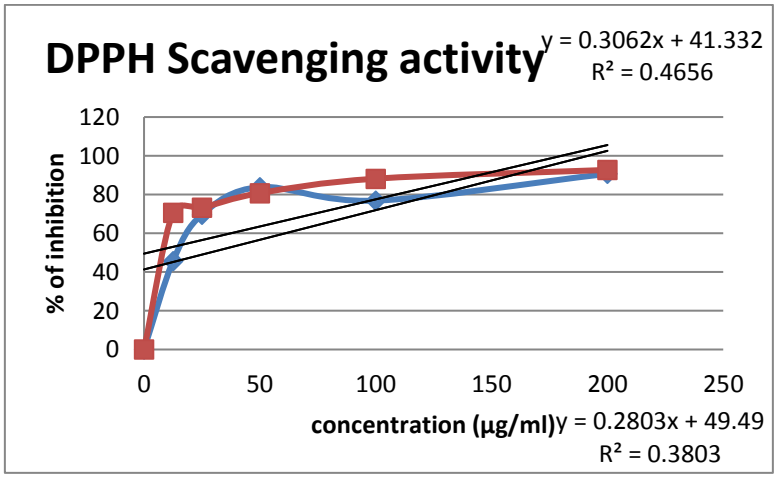


Fig 4.2.1 : Graphical Representation of DPPH Scavenging Activity test where red line is for sample and blue line is for standard

Table 4.2.1 : DPPH Scavenging activity for Methanolic extract of *G.cowa* Leaf :

Concentration (µg/ml)	Leaf methanol Absorbance	% of inhibition of Leaf methanol
0	0	0
12.5	0.468	45.5814
25	0. 261	69.65116
50	0.139	83.83721
100	0.08	76.86047
200	0.058	90.69767

Table 4.2.2 : DPPH Scavenging activity of Ascorbic acid :

Concentration (µg/ml)	Standard Absorbance	% of inhibition of Standard
0	0	0
12.5	0.399	70.66349
25	0.352	73.25201
50	0.233	80.74978
100	0.766	88.18804
200	0.24	92.7105

Table 4.2.3 :Determination of IC_{50} :

Methanol extract of G.cowa leaf /Ascorbic acid	**Regression Line**	**R^2 Line**	**IC_{50} Value (µg/ml)**
Methanol extract of *G.cowa* leaf	Y=0.3062x+41.332	R^2=0.4656	28.30
Ascorbic acid	Y=0.2803x+49.49	R^2=0.3803	1.8194

4.3 Total Phenolic content Test :

Phenolic compound of plant acts as primary antioxidants or free radical scavenger . *Garcinia cowa* leaf extract in methanol showed high concentration of total phenolic content.

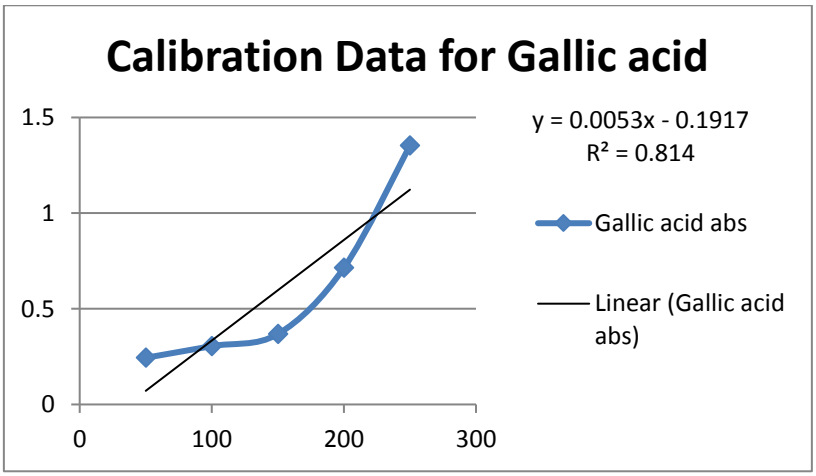


Fig 4.3.1: Total Phenolic content 223.1333±24.5375 (mg/g) gallic acid equivalent
Results are expressed as mean ± standard deviation where the x=3.

Table 4.3.1: Absorbance Data of Total phenolic content test :

Test Tube No.	Leaf methanol
1.	0.996
2.	0.995
3.	0.783

Table 4.3.2 : Determination of Mean and Standard deviation :

Methanolic extract of *G.cowa* leave Absorbance	Y=0.005x-0.191	Mean	Standard Deviation	Total phenolic content (mg/g)
0.996	237.4			
0.995	237.2	223.1333	24.53759	223.1333±24.5375
0.783	194.8			

4.4 Result of Anti-microbial Activity test :

Garcinia cowa leaf extract showed significant level of antimicrobial activity with respect to the Standard Kanamycin disc.The zone of inhibition was measured in mm. The maximum zone of inhibition was measured ranged from 9mm-24mm. The highest inhibition was observed against *Bacillus megaterium* (24mm). There were slight zone of inhibition were observed in case of several strains (7mm).

Table 4.4.1: Antimicrobial test (result in mm) :

Name of the strains	Zone of Inhibition (mm)		
	MeOH leaf extract of *G.cowa* (400µg/disc)	MeOH leaf extract of *G.cowa* (800µg/disc)	Standard Kanamycin (30 µg/disc)
Bacillus sereus	7	9	30
Bacillus megaterium	10	24	30
Bacillus subtillis	7	7	30
Salmonella paratyphi	7	7	30

Salmonella typhi	7	9	32
Vibrio parahemolyticus	7	8	35
Staphylocuccos aureaus	7	8	30
E. coli	7	8	35
Shigella dysenteriae	7	10	45
Pseudomonas aureaus	7	12	40

4.5 Discussion :

Plants have been used as medicine for millennia. Out of estimated 250 000 to 350 000 plantspecies identified so far, about 35 000 are used worldwide for medicinal purposes. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. Meanwhile, consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. The recent resurgence of plant remedies results from several factors: 1) the effectiveness of plant medicines; 2) the side effect of most modern drugs; and 3) the development of science and technology. It has been estimated that in the mid-1990s over 200 companies and research organizations worldwide are screening plant and animal compoundsfor medicinal properties. Actually, several important drugs used in modern medicine have come from medicinal plant studies, eg, taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide, teniposide, etc. As for drugs derived from orchids, some novel discoveries, both in phytochemical and pharmacological properties, were reported by some universities. However, studies on plants are very limited. Only about a third of the million or so species of higher plants have been identified and named by scientists. Of those named, only a tiny fraction has been studied. Nowadays the linking of the indigenous knowledge of medicinal plants to modern research activities provides a new approach, which makes the rate of discovery of drugs much more effective than with random collection.(Kong, J.M,2003).

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. In this study *Garcinia cowa* showed positive result in flavonoid test and steroid test. Flavonoids are a group of plant metabolites thought to provide health benefits through cell signalling pathways and antioxidant effects. These molecules are found in a variety of fruits and vegetables. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water.

Methanol extract of leaf of *Garcinia cowa* showed significant level of activity in DPPH scavenging test. The IC_{50} values were obtained by linear regression analysis of the dose response curves, which were plots of % inhibition versus concentration. The IC50 value for the sample was 28.30 whereas IC_{50} the standard is 1.8194. IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half. In this test we have found that the IC_{50} value of sample is much higher than the ascorbic acid which shows high DPPH scavenging activity.

Free radicals, generated in the human body as metabolic by-products or acquired from the environment, have been claimed to play a key role in affecting human health by causing oxidative damages associated with many degenerative diseases such as coronary heart diseases, atherosclerosis, aging, cancer and inflammatory conditions .In phenolic test the results showed that generally, the methanol extracts of leaf contained high levels of total phenolic contents. So this test also confirms about the antioxidant property.

Antimicrobial test is also done with the methanolic extract of leaf. Highest activity was observed against *Bacillus sereus (*9mm zone of inhibition) and *Bacillus megaterium* (24mm) at the concentration of 800µg/disc.So it shows effective activity against severe nausea, vomiting, food poisoning, diarrhea which is caused by *Bacillus sereus.* Antimicrobial activity against *Pseudomonas aureaus* is also found(12 mm) which is a causative agent of generalized inflammation and sepsis of tissues which have reduced immunity. Activity against *Shigella dysenteriae* (10mm) was also found which causes worldwide endemic form of bacillary dysentery. Activity against *Salmonella typhi* (9mm) was also monitored which is a causative agent of typhoid.

Garcinia cowa also have antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral activities. The pericarp of is a source of xanthones and other bioactive substances. Prenylated xanthones isolated from *G.cowa* have been extensively studied; some members of these compounds possess antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, antifungal and antiviral properties.

CHAPTER-5

Conclusion

5.1 Conclusion :

There are thousands of chemical constituent has been isolated from several parts of *Garcinia cowa* . Among the parts of this tree, leaves are the best source of metabolites which have been isolated, flavonoids, phloroglucinols ,xanthones & tanins. Some of these compounds show interesting pharmacological activities e.g anti-malarial, anti-microbial , anti-cancer properties. Methanolic extract of *Garcinia cowa* leaves possess strong antioxidant activity and anti-microbial activity. Significant amounts of flavonoids existed in its leaves, found in higher amount compared to other compounds. Therefore, supplementing a balanced diet with *Garcinia cowa* leaves may have beneficial health effects.The leaves could be beneficial in food preservation by applying it as a natural antioxidant in high-fat food products to delay lipid oxidation which is still under study.The whole plant is still under investigation with the prospect of identifying new bioactive compounds in the near future.

References:

Lichterman, B. L. , 2004 , "Aspirin: The Story of a Wonder Drug". *British Medical Journal*. 329 (7479) : 1408

Collins, Minta 2000 *Medieval Herbals: The Illustrative Traditions*. University of Toronto Press. p. 32.

Tapsell, L. C.; Hemphill, I.; Cobiac, L. et al. (August 2006). "Health benefits of herbs and spices: the past, the present, the future". *Med. J. Aust*. 185 (4 Suppl): S4–24

Billing, Jennifer,1998, P.W. (March 1998). "Antimicrobial functions of spices: why some like it hot". *Q Rev Biol*. 73 (1): 3–49.

Sumner, Judith ,2000, *The Natural History of Medicinal Plants*. Timber Press. p. 17

Carrubba, 2012 , Carrubba, A.; Scalenghe, R. (2012). "Scent of Mare Nostrum — Medicinal and Aromatic Plants (MAPs) in Mediterranean soils". *Journal of the Science of Food and Agriculture*. 92 (6): 1150–1170.

Kala, Chandra Prakash*; Sajwan (2007). "Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building". Current Science. 93 (6): 797–806.*

Cravotto G, 2010, Cravotto G.; Boffa L.; Genzini L.; Garella D. (February 2010). "Phytotherapeutics: an evaluation of the potential of 1000 plants". *J Clin Pharm Ther*. 35 (1): 11–48.

Nekvindová, J ,2007, Nekvindová, J.; Anzenbacher, P. (July 2007). "Interactions of food and dietary supplements with drug metabolising cytochrome P450 enzymes". *Ceska Slov Farm*. 56 (4): 165–73.

O'Connor ,2013, Anahad. "Herbal Supplements Are Often Not What They Seem". *The New York Times*. Retrieved 20 may 2017

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

Hossan, S., Agarwala, B., Sarwar, S., Karim, M., Jahan, R. and Rahmatullah, M., 2010. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. *Ethnobotany Research and Applications*, *8*, pp.061-074.

Rahmatullah, M., Mollik, A.H., Ahmed, N., Bhuiyan, Z.A., Hossain, M., Azam, N.K., Seraj, S., Chowdhury, M.H., Jamal, F., Ahsan, S. and Jahan, R., 2010. A survey of medicinal plants used by folk medicinal practitioners in two villages of Tangail district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, pp.357-363.

Cheek,M.,2004. *"Garcinia kola". IUCN Red List of Threatened Species. Version 2008. International Union for Conservation of Nature. Retrieved 21 may 2017.*

Dr. Chiranjit Parmer,2008 ,Frutipedia; Encyclopedia of edible Fruits around the world,originally uploaded May 2008, Revised 1-12-2013 [Available at http://www.fruitipedia.com/index.]. Retrieved 21 may 2017.

Likhitwitayawuid, K., Phadungcharoen, T. and Krungkrai, J., 1998. Antimalarial xanthones from Garcinia cowa. *Planta medica*, *64*(01), pp.70-72.

Xu, G., Kan, W.L., Zhou, Y., Song, J.Z., Han, Q.B., Qiao, C.F., Cho, C.H., Rudd, J.A., Lin, G. and Xu, H.X., 2010. Cytotoxic acylphloroglucinol derivatives from the twigs of Garcinia cowa. *Journal of natural products*, *73*(2), pp.104-108.

Ritthiwigrom, T., Laphookhieo, S. and Pyne, S.G., 2013. Chemical constituents and biological activities of Garcinia cowa Roxb.

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

WONG, L.P. & KLEMMER, P.J. (2008): Severe lactic acidosis associated with juice of the mangosteen fruit, *Garcinia mangostana*. *American Journal of Kidney Diseases* **51**(5): 829-833. doi:10.1053/j.ajkd.2007.12.043

Aziz, M.A. and Feeroz, M.M., 2009. Utilization of forest flora by Phayre's Leaf-Monkey Trachypithecus phayrei (Primates: Cercopithecidae) in semi-evergreen forests of Bangladesh. *Journal of Threatened Taxa*, *1*(5), pp.257-262.

World Conservation Monitoring Centre (1998). "Garcinia cadelliana". IUCN Red List of Threatened Species. Version 2008. International Union for Conservation of Nature. Retrieved 23 December 2008.

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

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

Xu, G., Kan, W.L., Zhou, Y., Song, J.Z., Han, Q.B., Qiao, C.F., Cho, C.H., Rudd, J.A., Lin, G. and Xu, H.X., 2010. Cytotoxic acylphloroglucinol derivatives from the twigs of Garcinia cowa. *Journal of natural products*, *73*(2), pp.104-108.

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.

Likhitwitayawuid, K., Phadungcharoen, T., Mahidol, C. and Ruchirawat, S., 1997. 7-O-Methylgarcinone E from Garcinia cowa. *Phytochemistry*, *45*(6), pp.1299-1301.

LIU, Y., QIU, Y.P., ZHANG, L. and CHEN, J., 2005. Dormancy breaking and storage behavior of Garcinia cowa Roxb.(Guttiferae) seeds: implications for ecological function and germplasm conservation. *Journal of Integrative Plant Biology*, *47*(1), pp.38-49.

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

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.

Siridechakorn, I., Phakhodee, W., Ritthiwigrom, T., Promgool, T., Deachathai, S., Cheenpracha, S., Prawat, U. and Laphookhieo, S., 2012. Antibacterial dihydrobenzopyran and xanthone derivatives from Garcinia cowa stem barks. *Fitoterapia*, *83*(8), pp.1430-1434.

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.

Wahyuni, F.S., Byrne, L.T., Dianita, R., Jubahar, J., Lajis, N.H. and Sargent, M.V., 2004. A new ring-reduced tetraprenyltoluquinone and a prenylated xanthone from Garcinia cowa. *Australian journal of chemistry*, *57*(3), pp.223-226.

Shen, J., Tian, Z. and Yang, J.S., 2007. The constituents from the stems of Garcinia cowa Roxb. and their cytotoxic activities. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *62*(7), pp.549-551.

Ritthiwigrom, T., Laphookhieo, S. and Pyne, S.G., 2013. Chemical constituents and biological activities of Garcinia cowa Roxb.

Liu, Y., Chen, J., Bai, Z., Deng, X. and Zhang, L., 2001. Seed dispersal, seed predation, and seedling spatial pattern of Garcinia cowa (Guttiferae). *Acta Phytoecological Sinica*, *26*(4), pp.427-434.

Sakunpak, A. and Panichayupakaranant, P., 2012. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. *Food Chemistry*, *130*(4), pp.826-831.

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

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

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.

Sarma, A., Sarmah, P., Kashyap, D. and Kalita, A., 2014. Evaluation of nutraceutical properties and antioxidant activity of Garcinia cowa Roxb. Ex Choisy fruits found in Assam (India). *World J. Pharm. Pharmaceut. Sci*, *3*, pp.853-853.

Kong, J.M., Goh, N.K., Chia, L.S. and Chia, T.F., 2003. Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica*, *24*(1), pp.7-21.