Determination of Cytotoxic Activity of Different Parts of *Garcinia Cowa.*

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

Submitted by:

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DECLARATION BY THE CANDIDATE

I, **Farjana Sarwar,** hereby declare that this dissertation entitled **"Determination of Cytotoxic Activity of Different parts of *Garcinia cowa*"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine and authentic research work carried out by me. The content 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, **"Determination of Cytotoxic Activity of Different parts of *Garcinia cowa*"** is a research work carried out by, **Farjana Sarwar** (ID: 2014-1-70-049) 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 on 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 **"Determination of Cytotoxic Activity of Different parts of *Garcinia cowa*"** is a research work carried out by **Farjana Sarwar** (ID: 2014-1-70-049), 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 **"Mother"** for her unconditional support and sacrifices.

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Abstract

Medicinal plants are defined as feral and/or cultivated plants that, based on tradition and literature records, can be directly or indirectly used for medical purposes. The basis for this use is that these plants contain so called active ingredients (active principles or biologically active principles) that affect physiological (metabolic) processes of living organisms, including human beings. The plant *Garcinia cowa* has been used for the general promotion of health and longevity. It is used as a traditional medicine for the treatment of various diseases. The bark is astringent; used in spasm. Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery. The aim of the present study is to evaluate the cytotoxic activity of the stem, bark and leaf of *Garcinia cowa*. Each samples were soaked in three different solvents (petroleum ether, dichloromethane and methanol). The brine shrimp lethality bioassay was performed to evaluate the cytotoxic activity of the *Garcinia cowa*. From this test, LC_{50} value of stem (109.95, 12.48, 70.63 μM), bark (110.23, 154.34, 17.09 μM) and leaf (48.73, 12.48, 30.19 μM) were calculated for petroleum ether, d_{ic}holoromethane and methanol solvents respectively. The R^2 value were approximately stem (0.829, 0.7086, 0.9395), bark (0.9161, 0.8903, 0.8522) and leaf (0.9172, 0.8813, 0.9516). So, it is evident that the stem, bark and leaf of *Garcinia cowa* are mild to moderate cytotoxic as well as biologically active. This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. Still there are plenty of scopes to establish a variety of properties which can be significantly beneficial to mankind.

Chapter One

Introduction

Introduction

The healing properties of plants were first discovered by chance, no doubt by early mankind in the quest of daily food. The observation that animals favored certain plants when they were injured or ill may have helped to guide primitive man in the search of cures for his ailments. Knowledge of the medicinal value of this plants was initially passed on verbally. Eventually, with the development with the society and written language, records on the use of medicinal plants were preserved in writing. (Capasso, 2003)**.** For thousands of years, medicinal plants have been used in various cultures of the world as a safe therapeutic modality. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores of people of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75-80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects. WHO consultative body of medicinal plants has formulated a definition of medicinal plants in the following way-

"A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs".
(Sofowora, 1982)

The operation of medicinal plants is based on the rich experiences of innumerable healers over centuries, inherited from ancestors, healer-to-healer transfer, or developed through personal experiences over time. Modernity or cultural revolutions have not altered the in-depth wisdom of this natural medical paradigm. Consequently, no modern system of medicine can ordinarily lay claim to it. The traditional system of treatment, differing in concept and protocol, exemplifies well-developed systems such as allopathic, homeopathic, ayurvedic, and Chinese systems of treatment. (Schippmann *et al*, 2002)

Most of the civilized nations have developed their own Materia Medica, compiling details about various plants used for therapeutic purposes. The merging of this human pharmacopoeia of natural origin with the incredible development in the various fields of modern medical sciences indeed provides the foundation for a much needed revolution in the existing health care system (Kesarwani *et al*, 2013) (Olurishe *et al*, 2014).

Extensive investigations have revealed that medicinal plants in different shapes, either in crude form or pure molecules isolated from them, represent the most ancient mode of medication. Archaeological studies have been provided reasonable evidences that the healing properties of plants were known to peoples in prehistoric time.6-8 Since the medicinal usage of the plants is as old as human civilization, some of the oldest references are available in the Artharvaveda, which is the basis of the traditional Indian medicine called ayurvedic medicine (dating back to 2000 BCE). Mesopotamians (1700 BCE)9 described the use of clay tablets, and there is documented evidence of the use of Eber Papyrus by Egyptians (1550 BCE).10 Other documented data that revealed the medicinal usage of plants are De Materia Medica, written by Dioscorides between CE 60 and 78, and Pen Ts'ao Ching Classic of Materia Medica, written around 200 CE.10. (Khan *et al*, 2012) (Ahmad *et al*, 2014).

1.2 Utilization of Medicinal Plants in Various Cultures

1.2.1 Greek period

Greek civilization was an epoch of science and philosophy. The Greeks have made worthy contribution in pharmaceutical sciences, especially in phytopharmaceuticals. Aristotle has described 500 crude drugs used in the cure of different pathological conditions. Hippocrates (460-337 BC) is considered as the father of allopathic medicine. He formulated the first scientific medical paradigm of treatment. He proposed that a large number of pathological conditions were due to disturbance in the normal physiology of human systems. The treatment was, therefore, based on the causes of the diseases to normalize the imbalance body systems. (Sykiotis et al, 2006). He has pointed out nearly 400 samples of medicinal substances from plant origin. Theophrastus (370-287 BC), a student of Aristotle, has also mentioned 500 crude drugs in his book. Another important

name is that of Claudius Galen Pergamum (modern-day Bergama, Turkey: 129-199). He prepared vegetable drugs using different extraction techniques called Galenicals and introduced the concept of pharmaceutical formulation to formulate stable and therapeutically effective drugs. (Newman *et al*, 2000). He wrote some 300 books on plants.

1.2.2 Traditional Chinese Medicine

Traditional Chinese Medicine represents one of the oldest systems of treatment. Traditional Chinese medicine is unique in theories, treatment, and therapies. This effective system of medicine has tremendous importance in the history of medicine and has now received global recognition due to its evidence basis approach (Patwardhan *et al*, 2005) (Buerki *et al*, 2007) This system is nearly free of external influence. Fu His (2953 BC) is considered as the pioneer of this system of medicine. The prescription of traditional Chinese medicine addresses those exogenous factors that are considered to be engaged in the pathology. Later, emperors Shen Nung and Hong Ti developed this system more significantly. Chinese pharmacopoeia Pen Tsao contained large numbers of remedies for various medical problems. Crown of written Chinese medicine goes to Shen Nong Ben Cao Jin (22-250 AD). CaoYuan Fang (550-630) wrote a book titled Zhu Bing Yuan Ji Lun, which described the etiology and symptoms of various diseases. This book is considered as a standard reference book for Chinese medical students. Wang Tao (702-772) has had an important contribution to traditional Chinese medicine. His published work Waitai Miyao described approximately 600 prescriptions. The foundation of his diagnostic philosophy was tongue. During different pathological conditions, the color and status of tongue changes. (Kopp *et al*, 2003) A great Chinese physician and naturalist, Li Shizen, has written a more inclusive pharmacopoeia Ben Ca Gang Mu, which was published in 1596. It has 1894 prescriptions and is still in use as reference and guide for research and schooling in China and several other communities. Importantly, traditional Chinese medicine was traditional knowledge that passed through generations, but only in the 1950s was it formatted in the form of academic educational training. (Xu *et al*, 2009)

1.2.3 Traditional Indian Medicine

Traditional Indian Medicine or ayurveda (known as the mother of all therapies) is considered as the oldest health care system on earth. The descriptions of the system are available in ancient literatures such as Rig-Veda and Atharva-Veda, approximately 5000 years BC. (Hsu *et al*, 2008) (Mukherjee *et al*, 2006). Ayurveda is a Sanskrit word that literary means knowledge of life. It is a natural healing system consisting of a mixture of physiologic and holistic medicine. Ayurveda defines man as a matrix of 7 basic tissues that works in harmony while disease is the outcome of imbalance in these components of the body. (Routh *et al*, 1999)

1.2.4 Arabic Period

The Arabs made enormous progress in the field of science and medicine after the fall of the Roman Empire. Scholars from the Islamic world translated books from Greece and Rome. Arab physicians introduced the concept of diet control and exercise along with medications. (Azaizeh *et al*, 2003). Arabs are actually the pioneers in the start of basic pharmacy practices. This includes the foundation of drug stores, the job description of physicians as diagnosticians of disease, and pharmacists being deputed for drug extraction and formulation. Due to this demarcation, the development in each field has started. As a result of this, Jaber Bin Hayan, a Muslim chemist, extracted and isolated various chemicals like alcohols, nitric acids, sulfuric acids, and so on. (Azaizeh *et al*, 2006). The religion of Islam has set a new breadth to the science of medicine in Arabia. Islam has specified means for a hygienic life style. These principles are primarily focused on Al Quran and Sunnnah and are titled as Tibb al-Nabi. (Qureshi *et al*, 2007). Ali Ibn Rabban Al Tabri (782-855 AD) was a renowned Muslim scientist. His book Firdous Al Hikmat, 11 consists of 7 parts in which one is specially focused on drugs and poisons. Abu Ali Al Hussan Ibn Sina (Avicenna, 980-932 AD) is the creator of the Greco-Arabic school of medicine. His book Canon was considered as a textbook on medicine in Europe, which describes more than 1000 drugs. His other book, Kitab Ash-Shifa, is considered as a scientific encyclopedia. Apart from the therapeutic and healing characteristics, the Arabs also described the toxic aspects of various plants. Abu Musa

Jabir ben Hayyan has written a very comprehensive book on different plant poisons and antidotes: The Book on Poisons and Antidotes. (Saad *et al*, 2006)

1.2.5 South American medicine

The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca and tobacco was common in these countries in the 14th and 15th centuries. (Sofowora, 1982).

1.3 Plants as a Basis of Some Important Drugs

Higher plants have been used as a source of drugs by mankind for several thousand years. In fact, ancient man was totally dependent on green plants for his day-to-day needs of medicaments. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. With the advancement of synthetic organic chemistry most of the active constituents of plants used in medicine were synthesized. At one time it was thought that ultimately all the plant drugs would be obtained from synthetic sources. However, in spite of phenomenal progress in the development of new drugs from synthetic sources and the appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw materials for some of the most important drugs. Although data are not available for all countries, a study carried out in the United States by Farnsworth and his colleagues between 1958 and 1980 indicated that although the number of prescriptions issued by community pharmacies in the United States increased considerably, the percentage of prescriptions containing one or more plant products 12 remained constant at a figure of 25%. It has been found that in highly developed countries like the United States more than 100 chemical constituents of definite structure derived from 41 species of plants were used in modern medicine. It has also been estimated that in addition to these active constituents, more than 96 crude extracts were also used in the United States. (Faried *et al*, 2000).

1.4 Characteristics of Medicinal Plants

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

Synergic Medicine:

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

Support of Official Medicine:

In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive Medicine:

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

1.5 Classification of medicinal plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Joy *et al*. 1998)

Table 1: classification of medicinal plants

Based on part used	1. Whole plant: diffusa, *Phyllanthus neruri* 2. Root: Dasamula 3. Stem:Tinospora cordifolia, Acorus calamus 4. Bark: *Saraca asoca* 5. Leaf: *Indigofera tinctoria, Lawsonia inermis, Aloe vera* 6. Flower:*Biophytum sensityvum, Mimusops elenji* 7. Fruit: *Solanum species* 8. Seed: *Datura stramonium*
Based on habitant	1. Tropical: *Andrographis paniculata* 2. Sub-tropical: *Mentha arvensis*

	3. Temperate: *Atropa belladonna*
Based on therapeutic value	1. Antimalarial: *Cinchona officinalis, Artemisia annua* 2. Anticancer: *Catharanthus roseus, Taxus baccata* 3. Antiulcer: *Azadirachta indica, Glycyrrhiza glabra* 4. Antidiabetic: *Catharanthus roseus, Momordica charantia* 5. Anticholesterol: *Allium sativum* 6. Antiinflammatory: *Curcuma domestica, Desmodium gangeticum* 7. Antiviral: *Acacia catechu* 8. Antibacterial: *Plumbago indica* 9. Antifungal: *Allium sativum* 10. Antiprotozoal: *Ailanthus sp., Cephaelis ipecacuanha* 11. Antidiarrhoeal: *Psidium gujava, Curcuma domestica* 12. Hypotensive: *Coleus forskohlii, Alium sativum* 13. Tranquilizing: *Rauvolfia serpentina* 14. Anaesthetic: *Erythroxylum coca* 15. Spasmolytic: *Atropa belladona, Hyoscyamus niger* 16. Diuretic: *Phyllanthus niruri, Centella asiatica* 17. Astringent: *Piper betle, Abrus precatorius* 18. Anthelmentic: *Quisqualis indica, Punica granatum* 19. Cardiotonic: *Digitalis sp., Thevetia sp.* 20. Antiallergic: *Nandina domestica, Scutellaria baicalensis* 21. Hepatoprotective: *Silybum marianum, Andrographis paniculata*

1.6 Functions that are provided by medicinal plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides, these plants play a critical role in the development of human cultures around the whole world. The high costs of western pharmaceuticals put modern health care services out of

reach for most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for first nations and immigrant populations, who have tended to retain ethnic medical practices. In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems.

The reasons for this interest are varied, and include:

- ✓ Low cost: herbals are relatively inexpensive and the cost of pharmaceuticals to government and individuals is rising.
- ✓ Drug resistance: the need for alternative treatments for drug-resistant pathogens
- ✓ Limitations of medicine: the existence of ailments without an effective pharmaceutical treatment
- ✓ Medicinal value: laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants
- ✓ Cultural exchange: expanding contact and growing respect for foreign cultures, including alternative systems of medicine
- ✓ Commercial value: growing appreciation of trade and other commercial economic opportunities represented by medicinal plants. (Czygan, 1990)

There are three main reasons for which plants have been found useful in medicine.

1. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents.
2. Second, they may be used as agents in the synthesis of drugs.
3. Finally, the organic molecules found in plants may be used as models for synthetic drugs. Historically, the medicinal value of plants was tested by trial and error, as in the Doctrine of Signatures.

Others

- ✓ Many of the modern medicines are produced indirectly from medicinal plants, for example: aspirin.

- ✓ Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- ✓ Many food crops have medicinal effects, for example garlic.
- ✓ Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species.3
- ✓ Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- ✓ Cultivation and preservation of medicinal plants protect biological diversity, for example: metabolic engineering of plants.

1.7 Medicinal plants and Bangladesh

Bangladesh is very rich in Bio-diversity. It has more than 500 medicinal plants species. An alarmingly populous, but size-wise a very small country is rather unique in having diversified genetic resources in a wide range of habitats. Increasing population pressure and multifarious anthropogenic activities on the natural ecosystems are posing severe and serious threats to once dense and rich genetically diversified plant communities of this country. Loss of habitats from the wild forests as well as from the village groves, cultivated plains and wild lands are quite common in this country. A broad genetic base has been replaced by a narrow one, and the old genetic diversity is disappearing both inside and outside of the ancient gene centers. This trend is inevitable with the need for highly efficient and uniform cultivars in advanced and sophisticated farming systems. At present, we have no real protected area for natural genetic resources and also have no specific practical policy on conservation of biodiversity. Although there are several gene banks having limited facilities to preserve some economic crops like rice, jute, wheat, pulses etc in Bangladesh, but there is no centralized organization to maintain germplasms of the wild relatives for agriculture, horticulture, medicinal and economically less important forest species. Bangladesh Agricultural Research Council (BARC) is very worried about this. However, the rich and diverse heritage of traditional medicinal system in the Indian subcontinent including Bangladesh is increasingly threatened by the interplay of a number of factors such as rapid deforestation and habitat destruction, indiscriminate collection and exploitative trade network. In Bangladesh there are about

297 Unani, 204 Ayurvedic and 77 Homeopathic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi– processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations (Ullah *et al*,2012)

Table 2: Some Medicinal Plants of Bangladesh (Rahman, 2013)

Serial no	**Common name**	**Botanical name**	**Parts used**
1	Malkunki	*Celustrus paniculatus*	Wild Bark, leaves, seed
2	Hajodi	*Cissusqua drangularis L.*	Whole plant
3	Khira	*Cucumis sativus L.*	Fruit, seed
4	Gudmar	*Gymnema sylvestre Retzx*	Whole plant, leaves
5	Satavar	*Asparagus adscendens Roxb*	Tubers
6	Safedmusli	*Chlorophytum borivilianum*	Tubers
7	Puskarmul	*Inular acemosa Hook*	Roots
8	Sakarkhand	*Manihotes culentacrantz*	Tubers
9	Cockscomb	*Celosia cristala L.*	Inflorescence
10	Red poppy	*Papaver rhoeas*	Flowers
11	Caper spurge	*Euphorbia lathyrus*	Seed latex
12	Kalazira	*Nigella sativa L.*	Seed
13	Afim	*Papaver somniferum L.*	Latex, seed
14	Pipli	*Piper Longum L.*	Fruits, roots
15	Babchi	*Psorale acorylifolia*	Seed, Fruit
16	Bael	*Aeglemar melos L. Corr.*	Roots, leaves, fruit
17	Neerh	*Azaflirachta indica*	Bark leaves, flowers, seed, oil
18	Palas	*Buteamonos sperma (Lam.)*	Bark, leaves, flowers, seed, gum

19	Gugul	*Commiphoram ukul Engh J*	Resinous gum
20	Olive	*Olea europeae*	Leaves, oil
21	Arjun	*Terminalia arjuan Roxb.*	Bark
22	Behela	*Terminalia bellirica Gaertu*	Bark, fruit
23	Hirda	*Terminalia bellirica Gaertu*	Fruits
24	Nagakesar	*Mesua ferrea L.*	Blowers, oil .
25	Markingnut	*Semecarpus anacardium L.*	Fruits

1.8 Traditional Medicine

Bangladesh possesses a rich flora of medicinal plants. Out of the estimated 5000 species of different plants growing in this country more than a thousand are regarded as having medicinal properties. Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Because of their potentialities and close association with the culture and tradition of the people, traditional systems of medicine have assumed a unique position in the health care of the people living in even the remotest areas of the country. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts (Majumdar *et al*, 2006). Among the largest ethnic group, the bangles on the main land, there are two distinct forms of traditional medicine practice.

One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:

- ✓ **Folk medicine**, which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like bloodletting, bone-setting hot and cold baths, therapeutic fasting and cauterization.

- ✓ **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods etc.
- ✓ **Spiritual medicine**, which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

The other is the improved and modified form based on the following two main traditional systems:

- ✓ **Unani-Tibb or Graeco-Arab system**, which has been developed by the Arab and Muslim scholars from the ancient Greek system, and
- ✓ **Ayurvedic system,** which is the old Indian system, based on the Vedas the, oldestscriptures of the Hindu saints of the Aryan age (World Health Organization, 2000).

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them. As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field. Some crude drugs used as medicine in Bangladesh are reported in following table.

Table 3: Some crude drugs used as medicine in Bangladesh (Ali, 1991)

Scientific Name	Plant Part(s) Used	Treatment
Callicarpa japonica	Leaf	Dyspepsia, heart burn
Callicarpa macrophylla	Whole plant	Tonic, dermatitis, cancer, antidote.
Clerodendrum indicum	Whole plant	Rheumatoid arthritis, jaundice, skin diseases.

Clerodendrum inerme	Leaf, flower	Night blindness, pneumonia, colic, rheumatoid arthritis.
Clerodendrum trichotomum	Leaf, stem, flower	Heart diseases, rheumatoid arthritis, skin diseases.
Clerodendrum viscosum	Whole plant, leaf	Giddiness, typhus, colic in cattle, diabetes, fever, aphrodisiac.
Lantana camara	Root, flower	Cough, mental diseases, fever.
Stachytarpheta indica	Leaf, stem	Leukorrhea.
Premnain tegrifolia	Leaf, bark, root	Fever, energy stimulant
Lippia alba	Leaf	Cuts and wounds.

1.9 Tribal medicine

In different localities of Rangamati and Bandarban Districts of Bangladesh a survey was carried out between 2001 and 2002 to document medicinal plants. A total of 69 medicinal plants under 40 families were documented during this work, which the tribal use to treat about 50 diseases (Yusuf *et al*, 2006).

Some examples are given below-

Table-4: Some tribal medicinal plants & their uses (Yusuf *et al*, 2006)

Scientific Name	Tribal name	Locality	Disease
Annona mouricata L.(Annonaceae)	Marma Penchi	Hangshamapara Bandarban	Pain in head and leg
Kalanchoe pinnata (Crassulaceae)	Tanchongya-Rockkia	Naramuk Rajasthali	Cough and asthma of children
Leea indica (Leeacea)	Chakma Haskura	Toolaban Marissa	Sore, leprosy, eczema, itching, bone fracture
Croton Caudatus Geisel (Euphorbiaceae)	Chakma sholokjara	Toolaban Marissa	Arthritis, paralysis

Eupatorium odoratum (Asteraceae)	Tonchongya Demrapata gach	Naramuk, Rajsthali	Bleeding

1.10 Resource of natural products for establishment of new drugs

Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species. Nature has been a source of therapeutic agents and a significant number of modern drugs have been developed from natural sources, many based on their use in traditional medicine. Over the last century, a remarkable number of top selling drugs have been derived from natural products (Vincristine from *Catharanthus roseus*, morphine form *Papaver somniferum*, quinine and quinidine from *Cinchona* spp.) Nowadays, approximately 40% of the modern drugs have been developed from natural source. More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anti –cancer drugs were from natural origin. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancer had natural origin. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporine and paclitaxe).

l Of the 30 top - selling medicines were natural products or their derivatives (Nauman, 2007) In light of all these facts, natural product drug discovery process failed to generate little respect. As drug discovery has emerged into a highly competitive era in which the quality of chemical collections and the time taken from assay to drug development are crucial factors in the success of a company, combinatorial chemistry has become the darling of the pharmaceutical industry, bringing with it the promise of new level of chemical diversity (Strohl, 2000). But this adoption of new strategy by the pharmaceutical companies gained little momentum. Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering and met genomic approaches to identify novel natural product lead molecules have met with limited success. These disappointments have led the pharmaceutical industry to consider whether natural product chemical diversity can or will continue to generate valuable templates for drug development.

Natural products offer a potentially infinite source of chemical diversity unparalleled to any synthetic chemical collection or combinatorial chemistry approach. In addition to that, these potent natural product compounds can have astounding chemical structures that can lead to unexpected, alternative medicinal chemistry programs based on important biological targets (Strohl, 2000). In the past few years, new natural products with a wide variety of chemical classes have been reported in the scientific literatures. Moreover, a total of 19 natural product based drugs were approved for marketing worldwide in between the year 2005 to April 2010, among which 7 being classified as natural products, 10 semi-synthetic natural products and 2 natural product derived drugs. (Mishra *et al*, 2011)

1.11 Approaches of drug development

The major portion of the present day knowledge of the medicinal properties of plants is the sum total of some observations and experiences. According to some generous estimates, almost 80 percent of the present day medicines are directly or indirectly obtained from plants. (Ghani, 2012)

1.11.1 Steps of drug development from plant sources given below:

1.11.1.1 Selection of plant species:

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

1.11.1.2 Evaluation of toxicity:

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

1.11.1.3 Preparation of plant sample and element analysis:

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

1.11.1.4 Biological Testing:

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

1.11.1.5 Isolating active compounds:

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

1.11.1.6 In-vivo analysis:

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

1.11.1.7 Commercialization:

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

1.12 Extraction of crude from medicinal plants

1.12.1 Extraction procedures

There are several extraction procedures for obtaining active component from medicinal plants.

1.12.2 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture

then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

1.12.3 Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

1.12.4 Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

1.12.5 Decoction

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

1.12.6 Ultrasound extraction (Sonication)

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

1.13 Parameters for selecting an appropriate extraction method

1.13.1 Nature of constituents

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

1.13.2 Solvents used in extraction process

Solvent is very essential for crude extraction. Extraction largely depends on choice of solvent.

Solvents are chosen depends on various parameters:

- Polarity
- Solubility
- Density
- nature of plant
- chemical constituent
- miscibility
- dispersion coefficient

1.14. Cancer

Cancer remains one of the leading causes of morbidity and mortality globally. Amongst the non-communicable diseases, cancer is the second leading cause of death, after cardiovascular disease. (World Health Oganization, 2005). Cancer is responsible for one in eight deaths worldwide—more than AIDS, tuberculosis, and malaria together (Sener *et al*, 2005). Overall cancer incidence and mortality are higher in North America, Australia, New Zealand and Western Europe compared to the rest of the world. In the United States, one in four deaths is attributed to cancer (Jemal, 2010). Globally, the number of cancer deaths is projected to increase from 7.1 million in 2002 to 11.5 million in 2030. (Mathers, 2006).

1.14.1 Types of cancer:

Table 5: type of cancer and affected area

Type of cancer	Affected area
Anal cancer	Anus
Bone marrow cancer	Shafts of long bones

Colon cancer	Colon
Cervical cancer	Cervix
Eye cancer	Eye
Gynecological cancer	Female reproductive organs
Lung cancer	Lung
Osteo cancer	Metaphyseal region of tubular long bones
Wilms tumour	Kidney
Leukemia	Blood
Larynx cancer	Larynx
Testicular cancer	Testis
Rectal cancer	Rectum
Bladder cancer	Urinary bladder

1.14.2 Treatment strategies

There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.

- Induction chemotherapy is the first line treatment of cancer with a chemotherapeutic drug. This type of chemotherapy is used for curative intent. (Airley, 2009)
- Combined modality chemotherapy is the use of drugs with other cancer treatments, such as surgery, radiation therapy, or hyperthermia therapy.

- Consolidation chemotherapy is given after remission in order to prolong the overall disease-free time and improve overall survival. The drug that is administered is the same as the drug that achieved remission.

- Intensification chemotherapy is identical to consolidation chemotherapy but a different drug than the induction chemotherapy is used.

- Combination chemotherapy involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side-effects. The biggest advantage is minimising the chances of resistance developing to any one agent. Also, the drugs can often be used at lower doses, reducing toxicity

- Neoadjuvant chemotherapy is given prior to a local treatment such as surgery, and is designed to shrink the primary tumor. It is also given to cancers with a high risk of micrometastatic disease.

- Adjuvant chemotherapy is given after a local treatment (radiotherapy or surgery). It can be used when there is little evidence of cancer present, but there is risk of recurrence. It is also useful in killing any cancerous cells that have spread to other parts of the body. These micrometastases can be treated with adjuvant chemotherapy and can reduce relapse rates caused by these disseminated cells.

- Maintenance chemotherapy is a repeated low-dose treatment to prolong remission.

- Salvage chemotherapy or palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, in general, a better toxicity profile is expected.

1.14.3 Chemotherapy

Chemotherapy is routinely used for cancer treatment. Since cancer cells lose many of the regulatory functions present in normal cells, they continue to divide when normal cells do not. This feature makes cancer cells susceptible to chemotherapeutic drugs. Approximately five decades of systemic drug discovery and development have resulted

in the establishment of a large collection of useful chemotherapeutic agents. However, chemotherapeutic treatments are not devoid of their own intrinsic problems. Various kinds of toxicities may occur as a result of chemotherapeutic treatments. For example, 5-fluorouracil, a common chemotherapeutic agent, is known to cause myelotoxicity, cardiotoxicity (Avni *et al*, 2008) and has even been shown to act as a vasospastic agent in rare but documented cases (Rastogi *et al*, 1993). Another widely used chemodrug, doxorubicin causes cardiac toxicity (Aviles *et al*, 1993), renal toxicity and myelotoxicity. Similarly, bleomycin a wellknown chemotherapeutic agent, is known for its pulmonary toxicity (Karam *et al*, 1995) In addition, bleomycin shows cutaneous toxicity. Cyclophosphamide, a drug to treat many malignant conditions, has been shown to have bladder toxicity in the form of hemorrhagic cystitis, immunosuppression, alopecia and at high doses cardiotoxicity (Fraiser *et al*, 1991) The toxicity of chemotherapeutic drugs sometimes creates a significant problem in the treatment of cancer using allopathy or established medicine. Various therapies have been propounded for the treatment of cancer, many of which use plant-derived products. There are four classes of plant-derived anticancer agents in the market today, the vinca alkaloids (vinblastine, vincristine and vindesine), the epipodophyllotoxins (etoposide and teniposide), the taxanes (paclitaxel and docetaxel) and the camptothecin derivatives (camptotecin and irinotecan). Plants still have enormous potential to provide newer drugs and as such are a reservoir of natural chemicals that may provide chemoprotective potential against cancer.

1.14.4 General Toxicities of Antineoplastic / Anticancer Drugs are:

- Bone marrow depression
- Lymphocytopenia
- GIT Stomatitis
- Diarrhoea
- Nausea and Vomiting
- Alopecia
- Hyperuricaemia
- Hair loss

Antineoplastic drugs / Anticancer Drugs are not only used prominently in different types of cancers but also in conjunction with surgery, radiotherapy and immunotherapy in the combined modality approach for many solid tumors, especially metastatic.

1.14.5 Classification of Antineoplastic Agents / Anticancer Drugs

1.14.5.1. Alkylating Agents

- Nitrogen mustards: Melphalan, Cyclophosphamide, Ifosfamide
- Nitrosoureas
- Alkylsulfonates
- Ethyleneimines
- Triazene
- Methyl Hydrazines
- Platinum Coordination complexes: Cisplatin, Carboplatin, Oxaliplatin

1.14.5.2. Antimetabolites

- Folate Antagonists: Methotrexate
- Purine antagonists
- Pyrimidine antagonists: 5-Fluorouracil, Cytarabibe

1.14.5.3 Natural Products

a. Plant Products

- Vinca Alkaloids: Vincristine, Vinblastine
- Taxanes: Paclitaxel, Docetaxel
- Epipodophyllotoxins: Etoposide
- Camptothecins: Irinotecan

b. Microorganism Products

- Antibiotics: Doxorubicin, Bleomycin

- Enzymes: L-Asparaginase

1.14.5.4 Miscellaneous

- Hydroxyurea
- Imatinib Mesylate
- Rituximab
- Epirubicin
- Bortezomib
- Zoledronic Acid
- Geftinib
- Leucovorin
- Pamidronate
- Gemcitabine

1.14.5.5 Hormones and Antagonists

- Corticosteroids: Prednisone, Dexamethasone
- Estrogens: Ethinyloestradiol
- Antiestrogens: Tamoxifen
- Progesteron derivative: Megestrol Acetate
- Androgen: Testosterone propionate
- Antiandrogen: Flutamide , Bicalutamide
- Aromatase inhibitor: Letrozole, Anastrazole
- 5-alpha reductase inhibitor: Finasteride
- GnRH Analogue: Leuprolide, Buserelin
- Growth Hormone, glucagon and insulin inhibitor: Octreotide

1.14.6 Limitations

Chemotherapy does not always work, and even when it is useful, it may not completely destroy the cancer. Patients frequently fail to understand its limitations. In one study of patients who had been newly diagnosed with incurable, stage 4 cancer, more than two-thirds of patients with lung cancer and more than four-fifths of patients with colorectal cancer still believed that chemotherapy was likely to cure their cancer. (Weeks, 2012). The blood–brain barrier poses a difficult obstacle to pass to deliver chemotherapy to the brain. This is because the brain has an extensive system in place to protect it from harmful chemicals. Drug transporters can pump out drugs from the brain and brain's blood vessel cells into the cerebrospinal fluid and blood circulation. These transporters pump out most chemotherapy drugs, which reduces their efficacy for treatment of brain tumors. Only small lipophilic alkylating agents such as lomustine or temozolomide are able to cross this blood–brain barrier. (Gerstner, 2007). Blood vessels in tumors are very different from those seen in normal tissues. As a tumor grows, tumor cells furthest away from the blood vessels become low in oxygen (hypoxic). To counteract this they then signal for new blood vessels to grow. The newly formed tumor vasculature is poorly formed and does not deliver an adequate blood supply to all areas of the tumor. This leads to issues with drug delivery because many drugs will be delivered to the tumor by the circulatory system.

1.14.7 Resistance

Resistance is a major cause of treatment failure in chemotherapeutic drugs. There are a few possible causes of resistance in cancer, one of which is the presence of small pumps on the surface of cancer cells that actively move chemotherapy from inside the cell to the outside. Cancer cells produce high amounts of these pumps, known as p-glycoprotein, in order to protect themselves from chemotherapeutics. Research on p-glycoprotein and other such chemotherapy efflux pumps is currently ongoing. Medications to inhibit the function of p-glycoprotein are undergoing investigation, but due to toxicities and interactions with anti-cancer drugs their development has been difficult. Another mechanism of resistance is gene amplification, a process in which multiple copies of a gene are produced by cancer cells. This overcomes the effect of drugs that reduce the

expression of genes involved in replication. With more copies of the gene, the drug cannot prevent all expression of the gene and therefore the cell can restore its proliferative ability. Cancer cells can also cause defects in the cellular pathways of apoptosis (programmed cell death). As most chemotherapy drugs kill cancer cells in this manner, defective apoptosis allows survival of these cells, making them resistant. Many chemotherapy drugs also cause DNA damage, which can be repaired by enzymes in the cell that carry out DNA repair. Upregulation of these genes can overcome the DNA damage and prevent the induction of apoptosis. Mutations in genes that produce drug target proteins, such as tubulin, can occur which prevent the drugs from binding to the protein, leading to resistance to these types of drugs. Drugs used in chemotherapy can induce cell stress, which can kill a cancer cell; however, under certain conditions, cells stress can induce changes in gene expression that enables resistance to several types of drugs. (Moschovi, 2015)

1.14.8 Medicinal plants and cancer

The anticancer properties of plants have been recognized for centuries. Isolation of podophyllotoxin and several other compounds (known as lignans) from the common mayapple (*Podophyllum peltatum*) ultimately led to the development of drugs used to treat testicular and small cell lung cancer (Pettit *et al*, 1995). The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities. Among them, about 3,000 plant species have demonstrated reproducible anticancer activity.

Many studies have focused on the chemoprotective properties of plants such as anticarcinogenic properties of *Abrus precatorius* on Yoshida sarcoma in rats, fibrosarcoma in mice and ascites tumor cells (Reddy *et al*, 1969). Similarly, Dhar *et al*. have examined the anticancer properties of *Albizzia lebbeck* on sarcoma in mice and *Alstonia scholaries* on benzo[a]pyrene-induced forestomach carcinoma in humans (Dhar *et al*, 1968). Other plants that have shown anticarcinogenic properties include *Anacardium occidentale* in hepatoma, *Asparagus racemosa* in human epidermoid carcinoma, *Boswellia serrata* in human epidermal carcinoma of the nasopharynx, *Erthyrina suberosa* in sarcoma, *Euphorbia hirta* in Freund virus

leukemia, *Gynandropis pentaphylla* in hepatoma, *Nigella sativa* in Lewis lung carcinoma, *Peaderia foetida* in human epidermoid carcinoma of the nasopharynx, *Picrorrhiza kurroa* in hepatic cancers, and *Withania somnifera* in various tumors (Dhar, 1968) The anticancer characteristics of a number of plants are still being actively researched and some have shown promising results. **Some plants and plant products that have shown promise as anticancer agents are discussed in detail in the following sections**.

1.14.8.1 *Tinospora cordifolia* (Wild) Miers

Tinospora cordifolia, also known as guduchi in Sanskrit, giloya in Hindi and heartleaf moonseed plant in English, is a bulky, smooth, climbing deciduous shrub lacking bristles (Fig. 1). The most commonly used part of the shrub is the stem, but roots are also known to contain important alkaloids. This shrub is commonly found in India, Myanmar, Sri Lanka and China.

Figure 1: *Tinospora cordifolia*

Tinospora cordifolia, also known as guduchi in Sanskrit, giloya in Hindi and heartleaf moonseed plant in English, under cultivation at experimental fields of IIIM, Jammu, India.

T. cordifolia effectively kills HeLa cells *in vitro*, suggesting its potential as an anticancer agent. A dose-dependent increase in cell death was observed in HeLa cells treated with *T. cordifolia* extract as compared to the controls (Jagetia *et al*, 1998). The anticancer activity of dichloromethane extract of *T. cordifolia* in the mice transplanted with Ehrlich

ascites carcinoma has been demonstrated. *T. cordifolia* extract showed a dose-dependent increase in tumor-free survival with highest number of survivors observed at 50 mg/kg dose (Jagetia, 2006)

1.14.8.2 *Ziziphus nummularia* Wight

Ziziphus nummularia, also known as bhukamtaka sukhsharanphala in Sanskrit, harbor in Hindi and wild jujube in English, is a thorny small bush or a divaricating shrub, with pale-purplish stems and or grey-velvety stipular prickles in pairs. The different parts of the plant that are used for medicinal purposes are root, bark, stem, flowers and seeds. This shrub is generally found in India, Pakistan, Afghanistan, Egypt, Iran, Iraq, and Israel.

Betulin and betulinic acid (chemical structures shown on next page) are present within the bark and stem of Z. *nummularia* and have been shown to have antitumor activity (Sarek et al, 2005) Betulinic acid glycosides produce differential cytotoxicity, such that cancer cell lines are more sensitive than normal cells (Gauthier, 2006) Similarly, betulinic acid, a naturally occurring pentacyclic triterpenoid, shows selective cytotoxicity against a variety of tumor cell lines. Betulinic acid has been suggested to induce apoptosis by generation of reactive oxygen species, inhibition of topoisomerase I, activation of the mitogen activated protein kinase (MAP kinase) cascade, inhibition of angiogenesis, and modulation of pro-growth transcriptional activators and aminopeptidase-N activity. Furthermore, betulinic acid has been shown to induce apoptosis by a p53- and CD95-independent mechanism. These mechanisms may be responsible for the ability of betulinic acid to effectively kill cancer cells that are resistant to other chemotherapeutic agents (Eiznhamer *et al*, 2004)

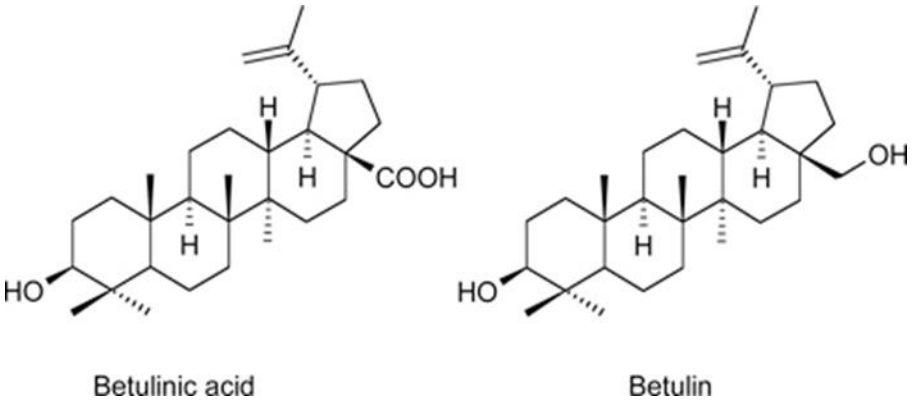


Figure 2: Betulinic acid and Betulin

It has been shown that combined treatment of betulinic acid and anticancer drugs, act in concert, to induce loss of mitochondrial membrane potential and the release of cytochrome *c* and second mitochondria derived activator of caspase (Smac) from mitochondria. These changes are suggested to result in the activation of caspases and induce apoptosis. Notably, betulinic acid augments the anticarcinogenic effect of different cytotoxic compounds of different modes of action (for example, doxorubicin, cisplatin, taxol, or actinomycin D). Importantly, betulinic acid potentiates the apoptotic effect of anticancer drugs in different tumor cell lines, including *p53* mutant cells, as well as primary tumor cells, but not in human fibroblasts indicating some tumor specificity.

1.14.8.3 *Andrographis paniculata* (Burm. F.) Nees

Andrographis paniculata, commonly known as bhunimba and kalmegha in Sanskrit, kiryat in Hindi and the king of bitters and chiretta in English, is found in India and Sri Lanka (Fig. 2). The parts of the plant generally used for medicinal purposes are the roots and the leaves. *A. paniculata* extract contains diterpenes, flavonoids and stigmasterols. The primary medicinal component of Andrographis is the diterpene andrographolide (chemical structure shown below). Andrographolide, described as a "diterpene lactone" due to its ring like structure, has a very bitter taste and has a colorless crystalline appearance. *Andrographis* leaves contain the highest concentration of andrographolide (~ 2.25%), while the seeds contain the lowest. (Siripong, 1992)

Figure 3: *Andrographis paniculata*

Andrographis paniculata commonly known as bhunimba and kalmegha in Sanskrit, kiryat in Hindi and the kreat king of bitters and chiretta in English, under cultivation at experimental fields of IIIM, Jammu, India.

A major chemical constituent of *A. paniculata*, andrographolide has also shown significant anticancer and immuno-stimulatory activities. The *in vivo* results conducted in immuno-competent Swiss albino mice, demonstrated that andrographolide significantly inhibits the cancer cell proliferation without showing any signs of toxicity in mice, even at relatively high doses. (Ajaya *et al*, 2004)

Figure 4: Andrographolide

1.14.8.4 *Centella asiatica* Linn

Centella asiatica, known as mandukaparni in Sanskrit, brahmamanduki in Hindi and asiatic pennywort in English, is another plant that has shown potential as an anticancer agent. This plant is commonly found in India, Australia, Pacific Islands, New Guinea, Malaysia, and Iran. The whole plant or its leaves are being traditionally used for their therapeutic properties. Partially purified fractions of *C. asiatica*, dose-dependently inhibited the proliferation of transformed cell lines, including Ehrlich ascites tumor cells and Dalton's lymphoma ascites tumor cells. However, practically no toxic effects were detected in normal human lymphocytes. Chemical structure of asiaticoside, a major component of *C. asiatica* is shown in the next page.

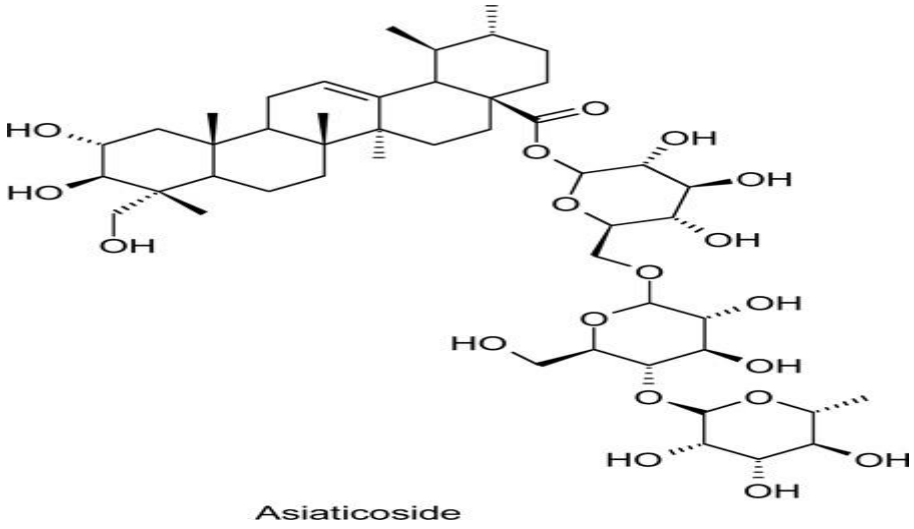


Figure 5: Asiaticoside

Partially purified fractions of *C. asiatica* also significantly suppressed the proliferation of mouse lung fibroblast cells in long-term culture. Oral administration of *C. asiatica* extracts slowed the development of solid and ascites tumors and increased the total life span of tumor-bearing mice. The mechanism underlying the antitumor activity of *C. asiatica* is suggested to be a direct inhibition of DNA synthesis. (Babu *et al*, 1995)

1.14.8.5 *Curcuma longa* Linn

Curcuma longa is popularly known as turmeric in English, haridra in Sanskrit and haldi in Hindi. The rhizome of the plant is traditionally used in cooking. The active ingredient

of this plant is curcumin (diferuloylmethane, chemical structure shown below), a polyphenol derived from the rhizome of the plant. Turmeric is used for both cancer prevention and treatment (Kuttan *et al*, 1985). The anticancer potential of curcumin is associated with its ability to inhibit proliferation in a wide variety of tumor cell types (Aggarwal *et al*, 2003) (Aggarwal *et al*,2003) (Shao *et al*, 2002). The anti-proliferative properties of curcumin may be related to its ability to down-regulate the expression of a number of genes, including NF-kappa B, Activator Protein 1 (AP-1), Epidermal growth receptor 1 (EGR-1), cycloxygenase 2 (COX2), lysyl oxidase (LOX), nitric oxide synthase (NOS), matrix metallopeptidase 9 (MMP-9), and tumor necrosis factor (TNF) (Surh *et al*, 2001).

Figure-6: *Curcuma longa*

Moreover, turmeric reduces the expression of various chemokines, cell surface adhesion molecules, cyclins and growth factor receptors, including epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2). In addition to its effects on gene expression, turmeric inhibits the activity of c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases (Aggarwal *et al*,2003) Turmeric has also been shown to inhibit tumor cell invasion and metastasis *in vitro* by reducing MMP-2 activity and by inhibiting HEp2 (epidermoid carcinoma cell line) cell invasion (Mitra *et al*, 2006)

Curcuma longa is popularly known as turmeric in English, haridra in Sanskrit and haldi in Hindi, under cultivation at experimental fields of IIIM, Jammu, India.

A number of studies have shown that curcumin induces apoptosis, inhibits proliferation and interferes with cell cycle progression is suggested to exert its anti-proliferative and apoptotic effects by inhibition of protein tyrosine kinase activity, inhibition of protein kinase C activity, suppression of c-myc mRNA levels and up-regulation of B-cell lymphoma 2 (Bcl-2) mRNA expression. Curcumin has been shown to cause apoptosis *in vitro* by bringing about a rapid decrease in mitochondrial membrane potential, release of cytochrome c, activatation of caspases 3 and 9, and downregulation of anti-apoptotic proteins Bcl-XL and Inhibitor of Apoptosis Protein (IAP). In LNCaP prostate cancer cells, curcumin was shown to increase apoptosis by enhancing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), promoting cleavage of pro-caspases 3, 8 and 9, and inducing cytochrome c release Recent studies also suggest that heat shock proteins may play a role in the induction of apoptosis by curcumin. (Rashmi *et al*, 2004)

Curcumin and its derivatives demonstrated significant inhibition of VEGF and bFGF-mediated corneal neovascularization and directly inhibited angiogenesis *in vivo* and *in vitro*. In addition to its antitumor effects *in vitro*, curcumin has been shown to prevent colon and gastric cancers in rodents. The mechanism underlying the protective effect of curcumin is suggested to be related to its ability to inhibit the growth of several tumor-associated and angiogenesis-associated genes Additionally, curcumin has been shown to inhibit the growth of nearly nineteen different strains of *H. pylori* (Mahady *et al*, 2002)

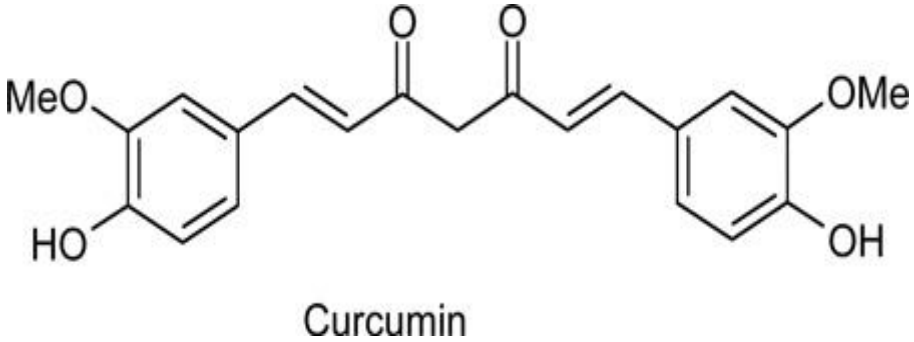


Figure 7: Curcumin

1.14.8.6 *Phyllanthus amarus* Schumach. & Thonn

Phyllanthus amarus is found in tropical Asia, especially in warmer parts of India and is known as bhumyamalaki in Sanskrit, jaramla in Hindi and stone breaker in English. The whole plant, leaves, roots and shoots are reportedly used for their medicinal values. *P. amarus* contains various lignans, flavanoids and tannins, and evidence suggests that *P. amarus* extract may exert antitumor effects. Oral administration of *P. amarus* extract significantly increased the life span and reduced tumor size in mice bearing Dalton's lymphoma ascites (DLA) and Erlich ascites carcinoma (EAC). The chemoprotective properties of this plant may be related to its ability to inhibit metabolic activation of carcinogenic compounds, induce cell cycle arrest and interfere with DNA repair. (Rajeshkumar, 2002)

The lignan-rich fraction of the hexane extract of *P. amarus*, and the various purified lignans namely nirtetralin (NIRT), niranthrin (NIRA), phyllanthin (PHYLLA), phyltetralin (PHYLT) (chemical structures shown below) have been reported to be effective in inhibiting P-gp (P-glycoprotein) function *in vitro*. Furthermore, these lignans, in combination with daunorubicin act as a Multiple Drug Resistance (MDR)–reversing agents.

1.14.8.7 Annona atemoya Mabb./*Annona muricata* Linn

Annona atemoya/muricata is a native of Caribbean, Central and South America. It is also commonly grown in South East Asia especially in eastern part of India. This plant is traditionally known as mamaphal in Hindi and sour-sop of America in English. The parts of the plant that are generally used for medicinal purposes are the root, bark, leaf and fruit. The fruit of *A. atemoya* contains bullatacin (chemical structure shown below), an acetogenin known to have antitumor properties. Bullatacin induces chromatin margination and tumor cell condensation, followed by apoptosis. *A. atemoya* contains two annomuricins namely A and B, which have shown cytotoxicity in human solid tumor cell lines A-549 lung carcinoma, MCF-7 breast carcinoma, and HT-29 colon

adenocarcinoma cell lines *A. atemoya* contains several other acetogenins that have also been shown to selectively induce cell death in tumor cells *in vitro*. In particular, two annonaceous acetogenins were found to produce cell death in the human hepatoma cell line HepG2 and hepatoma 2.2.15 cells (Chang *et al*, 2003)

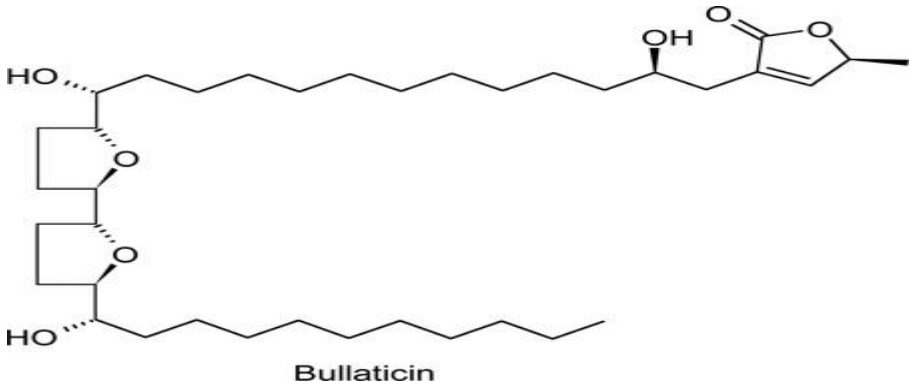


Figure 8: Bullaticin

1.15 Review on Mangosteen (*Garcinia Cowa*)

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

The fruit, capped by the prominent calyx at the stem end and with 4 to 8 triangular, flat remnants of the stigma in a rosette at the apex, is round, dark-purple to red-purple and

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

1.15.1 General Information

Family: Clusiaceae

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

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

English name: Cow Tree

1.15.2Taxonomical classification

Kingdom- Plantae

Phylum- Tracheophyta

Class- Magnoliopsida

Order- Malpighiales

Family- Clusiaceae

Genus- *Garcinia* L.

Species- *Garcinia cowa* Roxb

1.15.3 Lists of Plants under Garcinia genus

The plant list currently (July, 2017) contains 395 species including-

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

cambogia, Garcinia cantleyana, Garcinia celebica L.: (syn.: Garcinia benthamii), Garcinia cerasifer (H.Perrier), Garcinia clusiaefolia, Garcinia costata, Garcinia cymosa (K.Schum.), Garcinia decussate, Garcinia diversifolia, Garcinia dulcis – mundu, rata, Garcinia echinocarpa etc.

1.15.4 Distribution: Forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet. The genus Garcinia (Family: Clusiaceae) consists of over 200 species distributed in the tropics of the world chiefly in Asia, Africa, and Polynesia. They are evergreen polygamous trees, shrubs, and herbs. About 35 species are reported to exist in India, many of which are endemic and economically important with immense medicinal properties. (Roberts *et al,* 1984.)

1.15.5 Parts Used: Leaves, barks and fruits.

1.15.6 General Description

The fruits and young leaves are edible with a sour taste. The bark is dark brown with a yellow latex. The plant has unisex flowers: yellow orange female flowers found at the end of branches and male flowers found along the branches as clusters. The leaves are glossy, deep green, oblong and up to 6-15 cm in length and 2.5- 6.0 cm in width. The fruits are globose (2.5-6.0 cm in size), green when young and dull orange or yellow at maturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3- angled seeds. A medium-sized evergreen tree with horizontal branches and oval crown. Leaves 7.6-12.6 cm long, broadly to elliptically lanceolate, acuminate. Flower rather small, yellow; the male ones smaller in dense terminal clusters; the females 13 mm diameter or somewhat larger, solitary or by 3-5 at the end of the branchlets. Berry the size of a lime, slightly 6-8 lobed, dul red, somewhat depressed at the apex.

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.

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.

1.15.7 Plant Habitat

In India, species of *Garcinia* grow extensively in semiwild state, in the Konkan region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, and evergreen forests of Assam, Khasi, Jantia hills, Nagaland, West Bengal, and Gujarat.

1.15.8 Use

- In Malabar and Konkan regions of Southern India, they are used in garnishing curries and also as a replacement for tamarind.
- In North Eastern India, the sundried slices of the fruits are used for culinary purposes and as folk medicine. Some species like *Garcinia cambogia, G. indica,* and *G. cowa* are cultivated in certain parts of India. *G. pedunculata, G. kydia,* G. cowa, and *G. lanceaefolia* are the most important species in north eastern parts of India.
- Many species of Garcinia have fruit with edible arils and are eaten locally. The best-known species is the mangosteen (*G. mangostana*), which is now cultivated throughout Southeast Asia and other tropical countries.
- The seeds of *G. indica* fruits yield valuable edible fat known as kokum butter. The fruits of *Garcinia* are a food source for several animals. (CSIR, 1956.).
- Most species in *Garcinia* are known for their gum resin which is used as purgative or cathartic.
- Fruits of some *Garcinia* species are also one of the richest sources of red pigments in the plant kingdom.
- Fruit and syrup of *G. indica* are very popular in Konkan region and are antioxidant and antibacterial. (Negi, 2008)

Garcinia is the source for a natural diet ingredient (-) hydroxycitric acid. HCA (1,2dihydroxypropane-1,2,3-tricarboxylic acid) which is an anti-obesity compound is

present in the fruit rind and leaves of Garcinia and is known to inhibit lipid and fatty acid synthesis in living systems. HCA is also a hypocholesterolemic agent (Lowenstein, 1971). On a dry weight basis, HCA constitutes about 20–30% of the fruit. Lack of knowledge, coupled with habitat destruction, leads to genetic erosion of this forest resource and many species are threatened (Cheek, 2004). They need to be studied and conserved. Most Garcinia species occur only as natural populations and are known only locally. Many Garcinia species have edible arils and are eaten locally. Some species fruits are highly esteemed in one region but are unknown just a few hundred kilometers away. Perhaps, trees are cut due to lack of awareness and popularization of importance of Garcinia will help in conserving the local populations of this genus. Here it is worth to mention that the climatic parameters of both ecosystems are almost the same specially the altitude and rainfall pattern. The altitude varies from 100 to 600 MLS, while the annual rainfall varies from 1500 to 4500 mm.

1.15.9 Salient Feature of Family Clusiaceae

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

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

Scotch attorney, or cupey (Clusia rose), which is native to the Caribbean area, grows to about 10 metres (30 feet). It has leaves 10 cm (4 inches) long, flatly open flowers with six waxy, rosy-white petals, and many-seeded, multicelled, golfball-sized fruits. Like other species in the family, the fruits open and the valves spread widely like a star, exposing the succulent bright-orange tissue (arils) surrounding the seeds. Scotch attorney is planted as a beach shrub in areas exposed to salt spray.

C. grandiflora, which is native to Suriname, has larger flowers and ivory-white central stamen masses. Many members of the genus Clusia begin as epiphytes, or air plants, and

eventually send roots over the host tree to the ground. All of the 300 to 400 members of the genus are tropical American. Mammee Apple or mamey (*Mammea americana*), native to tropical America, produces a grapefruit-sized, rough, russet-skinned, edible fruit. The other members of the genus Mammea are tropical but especially common in Madagascar.

Figure 9: whole plant of *Garcinia Cowa*

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

1.15.10 Chemical Constituents and Biological Activities of *Garcinia cowa*

Many pharmaceutical drug discoveries originated from traditional folk medicine and its associated plant materials and bioactive secondary metabolites. The Genus Garcinia, belonging to the Family Clusiaceae which comprises about 300 species, have been widely investigated in terms of their bioactive ingredients. Native to Asia, Africa, South America and Polynesia, the plants are small to medium sized evergreen trees which may

grow up to 30 m in height and are widely distributed in the tropical and temperate regions of the world. Twenty-nine species have been observed in Thailand, with 20, 13, 12, 7, 6 and 3 species found in the south, middle, north, east, north-east and west of the country respectively. Garcinia is a rich source of secondary metabolites, especially triterpenes, flavonoids, xanthones and phloroglucinols. The latter two groups are well recognised as cheomotaxonomic markers for this genus. Many of the isolated compounds have a wide range of pharmacological activities including anticancer, anti-inflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant and antioxidant.

Garcinia cowa, commonly known as Cha-muang in Thai, is widely distributed throughout Malaysia, Thailand and Myanmar. The fruits and young leaves are edible with a sour taste. The bark is dark brown with a yellow latex. The plant has unisex flowers: yellow orange female flowers found at the end of branches and male flowers found along the branches as clusters. The leaves are glossy, deep green, oblong and up to 6-15 cm in length and 2.5- 6.0 cm in width. The fruits are globose (2.5-6.0 cm in size), green when young and dull orange or yellow at maturity with 5-8 shallow grooves, at least near the top, and contain 6-8 large 3- angled seeds. (Sharmin *et al*, 2004).

Figure 10: Flower of *Garcinia Cowa*

Figure 11: Fruit of *Garcinia Cowa*

Figure 12: Foliage of *Garcinia cowa*

Many parts of *G. cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an antifever agent while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant. The chemical composition and biological activities of various parts of *G. cowa* have been investigated. The major compounds found were xanthones and phloroglucinols. However, minor compounds. including depsidones, terpenoids, steroids and flavonoids,

were also observed. Currently, 78 compounds have been isolated from the twig, stem, fruit and latex.

1.15.11 Distribution and Biological Activity

The biological activities of the extracts from various parts of *G. cowa* have been investigated, including the hexane and chloroform extracts of the fruit rind and methanol extract of the leaves and twigs. The hexane and chloroform extracts from the fruit rind of *G. cowa* were tested against four Gram-positive bacteria *(Bacillus cereus*, *B. coagulans*, *B. subtilis* and *Staphylococcus aureus*) and one Gram-negative bacterium (*Escherichia coli*). Both extracts significantly inhibited bacterial growth of the Gram-positive bacteria ($_{IC50}$s 15-30 micro g/mL) but not E. coli (IC_{50}s 250-500 micro g/mL). The extracts were also found to inhibit the growth of Aspergillus flavus ATCC 46283, a common fungal food contaminant which produces aflatoxin B1. The degree of inhibition of aflatoxin B1 production (100% at a concentration of 2000 ppm) was found to be much higher than the inhibition of fungal growth (ca 40-60% at the same concentration). The methanol extracts of the leaves and twigs of *G. cowa* were evaluated for their ability to inhibit low-density lipoprotein peroxidation induced by copper ions. The twig extract had an IC_{50} value of 20.5 micro g/mL and was more potent (higher % inhibition at 1000 micro g/mL) than the leaf extract (IC_{50} not measured). The twig extract was more potent than the leaf extract on platelet aggregation of human whole blood induced by arachidonic acid, adenosine diphosphate and collagen. These activities may be due to the total phenolic content of these extracts, which were 19 and 61 mg of gallic acid equivalent per g of extract for the leaf and twig extracts respectively. (Schimmel *et al*, 2004).

Chapter Two

Literature review

2.1 Literature Review on *Garcinia cowa*

we have studied on stem, bark and leaf of this plant. Here is some literature review on different parts of this plant.

2.1.1. Cytotoxic Properties and Complete Nuclear Magnetic Resonance Assignment of Isolated Xanthones from the Root of *Garcinia cowa* Roxb.

Isolation of cytotoxic compounds from *Garcinia cowa* Cowanine is the active constituent from the roots of *Garcinia cowa* Complete nuclear magnetic resonance assignment of isolated compounds MS fragmentation of rubraxanthone. Phytochemical study on the roots of *G. cowa* yielded rubraxanthone (3), cowanine (4) and 1,5-dihydroxyxanthone (5). Compound 4 with an IC50 value of 4.1 ± 1.0 μM, 5.4 ± 2.3 μM and 11.3 ± 10.0 μM against MCF-7, H-460, and DU-145, respectively while compound 3 was found to be in active. The results indicate that *G. cowa* roots could be important sources of natural cytotoxic compounds. (Dachriyanus *et al*., 2016)

2.1.2 Cytotoxic compounds from the leaves of *Garcinia cowa* Roxb.

The aims of this study was to isolate compounds from the leaves of methanol extract of *Garcinia cowa* and to evaluated their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines. The dichloromethane fraction was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature these compounds were elucidated as methyl 2,4,6- trihydroxy-3-(3-methylbut-2-enyl)benzoate (1), garcinisidone-A (2) and methyl 4,6dihydroxy-2-(4-methoxy-5- (3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4-dienyloxy)-3-(3-methylbut-2-enyl)benzoate (3). Compound 1, 2 and 3 had IC50 value of 21.0 ± 10.2 μM, 21.2 ±8.4 μM and 17.2 ± 6.2μM against MCF-7, while only compound (2) was found to be in active against H-460 with IC50 value of 18.1 ± 6.7 μM. Conclusion: The results indicate that *G. cowa* leaves could be important sources of natural cytotoxic compounds and only compound (2) had activity against H-460 cell lines. (Wahyuni *et al.*, 2015)

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

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

2.1.4 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 9.10μM, respectively. On the other hand, compound 15 showed good cytotoxicity against HeLa cell with IC_{50} value of 9.34μM. (Kaennakam, Siripong and Tip-pyang, 2015)

2.1.5 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 dimericdihydrobenzopyran derivative, whereas the framework of 2 was a triprenyl caged-xanthoneprecursor. The antibacterial activities against Escherichia coli TISTR 780, Salmonellatyphimurium TISTR 292, Staphylococcus aureus TISTR 1466, and

methicillin-resistant S. aureus(MRSA) SK1 of the isolated compounds were also evaluated. Compounds 2 and 9 exhibitedgood antibacterial activity against MRSA SK1 with the same minimum inhibitory concentration(MIC) value of 2 μg/mL. Moreover, compound 2 also showed good antibacterial activityagainst S. aureus with an MIC value of 2 μg/mL. (Siridechakorn *et al*, 2012)

2.1.6 Cytotoxic acylphloroglucinol derivatives from the twigs of *Garcinia cowa.*

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

2.1.7 The constituents from the stems of *Garcinia cowa* Roxb. and their cytotoxic activities.

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

2.1.8 Two new xanthones from the stems of *Garcinia cowa.*

Twonewxanthones,1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl) xanthone (1) and 1, 5-dihydroxy-3-methoxy-6',6'-dimethyl-2H-pyrano(2',3':6,7)-4-(3-methylbut-2-enyl)xanthone (2), have been isolated together with six known xanthones:1,3,5-

trihydroxy-6',6'-dimethyl-2H-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),1,3,6,7tetrahydroxy xanthone (8), from the stems of *Garcinia cowa* (Guttiferae). (Shen and Yang, 2006)

2.1.9 Antibacterial tetraoxygenated xanthones from the immature fruits of *Garcinia cowa*.

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

2.1.10 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 IC50 values of 7.97 and 9.10 μM, respectively. On the other hand, compound 15 showed good cytotoxicity against HeLa cell with IC50 value of 9.34 μM. (Kaennakam, Siripong and Tip-pyang, 2015)

2.1.11 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. (Panthong *et al*, 2006)

2.1.12 Antioxidant and antiplatlet aggregation properties of bark extracts of Garcinia pedunculata and *Garcinia cowa.*

The bark extracts of *Garcinia pedunculata* and *Garcinia cowa*, which are abundant in the Northeastern regions of India, were screened for their antioxidant and in vitro antiplatelet aggregating activities. By β-carotene linoleate model for antioxidant assay, acetone extract of *G. pedunculata* and hexane extracts of *G. cowa* exhibited higher antioxidant activity (86.47 and 66.94 % respectively, at 25 ppm) than other extracts. Similar pattern was observed for superoxide radical scavenging method for antioxidant assay. The ethyl acetate extract of *G. pedunculata* and hexane extract of *G. cowa* exhibited higher antiplatelet aggregation capacity towards ADP induced platelet aggregation (IC_{50} 0.16 and 0.43 micro gram, respectively) than other extracts. (Sharma, Joseph and Singh, 2014)

2.1.13 Antimalarial xanthones from *Garcinia cowa.*

Five xanthones from the bark of *Garcinia cowa*, namely 7-O-methylgarcinone E (1), cowanin (2), cowanol (3), cowaxanthone (4), and beta-mangostin (5), were found to possess in vitro antimalarial activity against Plasmodium falciparum with IC_{50} values ranging from 1.50 to 3.00 micrograms/ml. Complete 1H- and 13C-NMR assignments of these compounds are also reported. (Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998)

2.1.14 Xanthones from *Garcinia cowa* Roxb. Latex.

Five xanthones named cowagarcinone A–E and six previously reported xanthones were isolated from the latex of *Garcinia cowa* Roxb. Their structures were determined on the basis of spectroscopic analysis. The crude latex and the isolated compounds were investigated for their radical scavenging activities. (Mahabusarakam, Chairerk and Taylor, 2005)

2.1.15 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, Jayaprakasha and Jena, 2008)*

2.1.16 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 B1 production 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 *et al*, 2005)

2.1.17 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 B1 production 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 *et al*, 2005)

2.1.18 Biphenyl and xanthone derivatives from the twigs of a *Garcinia* sp. (Clusiaceae)

The genus Garcinia is a wellknown rich source of bioactive xanthones and benzophenones. Some species of this genus also produce flavonoids and biphenyls as minor constituents. In this study, two new biphenyls, doitungbiphenyls A (1) and B (2), along with two biphenyls, schomburgbiphenyl (3) and nigrolineabiphenyl B (4); and four xanthones, 1,3,6-trihydroxy-8-isoprenyl-7-methoxyxanthone (5), morusignin K (6), 1,5-dihydroxyxanthone (7), and 1,7-dihydroxyxanthone (8), were isolated from the acetone extract of the twigs of a Garcinia sp. Their structures were characterized extensively by 1D and 2D NMR spectroscopy and HR-EI-MS. The cytotoxicity of the two new biphenyls against the oral cavity cancer (KB) and the breast cancer (MCF7) cell lines was also evaluated. (Siridechakorn *et al*, 2014)

2.1.19 Simultaneous determination of multi-class bioactive constituents for quality assessment of *Garcinia* species using UHPLC–QqQLIT–MS/MS.

Plants of genus Garcinia are therapeutically important and widely used in ayurvedic and traditional systems of medicine for the treatment of an array of ailments. In present study, a highly sensitive and efficient analytical method was developed and validated for rapid determination of twenty-six multi-class bioactive constituents in the leaf extracts of eleven Garcinia species using ultra high performance liquid chromatography-hybrid linear ion trap triple quadrupole mass spectrometry (UHPLC–QqQLIT–MS/MS). Chromatographic separation was accomplished on an Aquity UPLC BEH C18 column (50 mm × 2.1 mm id, 1.7 μm) using gradient elution of 0.1% formic acid in water and acetonitrile within 7.5 min. Quantitative analysis was carried out by multiple-reaction

monitoring mode (MRM) in negative electrospray ionization. The developed method was validated according to International Conference on Harmonization (ICH, Q2R1) guidelines. All standard calibration curves expressed good linear relationship ($r2 \geq 0.9991$) over the concentration range of 0.1–300 ng/mL. The precision was assessed by intra- and inter-day study which revealed RSD ≤ 1.93%. The recoveries of quantified compounds were between 95.45 and 104.43% with RSD ≤ 1.89%. This method was successfully applied to investigate quantitative variation among eleven Garcinia species. Moreover, principal component analysis (PCA) and hierarchical clustering analysis (HCA) were performed to compare and evaluate the quality of studied Garcinia species based on their quantitative data. The results indicated significant variation among eleven Garcinia species on the basis of twenty-six bioactive constituents. The developed method could be used as a tool for quality control and authenticity establishment of Garcinia species. (Pandeya *et al.*, 2015)

2.1.20 A comparative study on conventional and microwave-assisted extraction for microencapsulation of *Garcinia* fruit extract.

(−)-Hydroxycitric acid (HCA) is the principal acid present in the fruit rinds of certain species of Garcinia and is reported to have various health benefits. However, HCA is highly hygroscopic in nature and becomes lactonised during evaporation and drying. To reduce the lactonisation of HCA, *Garcinia cowa* fruit extract was obtained through two different extraction techniques, autoclave and microwave-assisted extractions, and was then microencapsulated using whey protein isolate (WPI) and denatured whey protein isolate (DWPI) with a 1:1 wall-to-core ratio using a spray drying technique. The microwave-assisted extracts encapsulated with WPI and DWPI had higher free HCA and net HCA recovery than the autoclaved extract encapsulated with similar wall materials. Furthermore, the microwave-assisted extracts and the associated encapsulated samples had higher antioxidant activity than their counterparts. The encapsulation of the microwave-assisted extracts with both wall materials had little variations in their free (55.04 and 54.58% for WPI and DWPI, respectively) and net HCA recovery (84 and 82%) and antioxidant activity (13.3 and 13.6%), which signified a smaller influence of

the wall materials. These results indicated that microwave-assisted extraction had a higher extraction efficiency, encapsulation efficiency and antioxidant activity with both wall materials. (Parthasarathi *et al.,* 2013)

2.1.21 The potential health benefit of polyisoprenylated benzophenones from *Garcinia* and related genera: Ethnobotanical and therapeutic importance.

The diversity present in biological activities and the medicinal significance of natural products provide a renewed interest in the use of natural compounds and, more importantly, their role as a basis for drug development. Advancements in the field of natural product chemistry provide valuable information on *Garcinia* fruits which revealed the presence of biologically important secondary metabolites named as polyisoprenylated benzophenones (PIBs). They are mainly present in the genus Garcinia (Guttiferae) which occupies a prominent position in the history of natural products. Compared to the long history of medicinal uses and widespread research on Garcinia, the study of polyisoprenylated benzophenones was relatively limited. During recent years, these PIBs have been recognized as interesting and valuable biologically active secondary metabolites as many of the isolated polyisoprenylated benzophenones exhibited significant cytotoxic activity in in vitro and in vivo assay. During past decades, some promising advances had been achieved in understanding the chemistry and pharmacology of polyisoprenylated benzophenones. However, there has been not any systematic review on the ethnobotanical importance, chemistry, isolation techniques, structure activity relationships and the biological activities of polyisoprenylated benzophenones. In this review, the biological activity of different structures of polyisoprenylated benzophenones isolated from genus *Clusia, Garcinia, Vismia, Allanblackia, Moronobea, Symphonia, Hypericum, Tovomita, Tovomiptosis and Ochrocarpus* have been described. Therefore, the goal of this review article would be a valuable reference for the natural product chemists and biologists working on these PIBs. Furthermore, the review article on polyisoprenylated benzophenones would also be useful from the drug discovery point of view as cytotoxic agents in near future. This review focuses our understanding about the specific biological effects of Garcinia fruits, which may be useful for predicting other

medicinal uses, potential drug or food interactions and may benefit people where the fruits are prevalent and healthcare resources are scarce. (Kumar, Sharma and Chattopadhyay, 2013)

2.1.22 Qualitative and quantitative analysis of polycyclic polyprenylated acylphloroglucinols from *Garcinia* species using ultra performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry.

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a group of natural products isolated from different Garcinia species with a wide range of important biological activities. In this study, an ultra performance liquid chromatography (UPLC) coupled to photodiode-array detection and quadrupole time-of-flight mass spectrometry (Q-TOF) method was developed to characterize 16 PPAPs in 10 Garcinia species. In source dissociation techniques based on cone voltage fragmentation were used to fragment the deprotonated molecules and multiple mass spectrometry (MS/MS) using ramping collision energy were used to further break down the resulting product ions. The resulting characteristic fragment ions were generated by cleavage of C1–C5 bond and C7–C8 bond through concerted pericyclic reaction, which is especially valuable for differentiating three types of PPAPs isomers. As such, two new PPAPs isomers present in minor amount in the extracts of *Garcinia oblongifolia* were tentatively characterized by comparing their tandem mass spectra to the known ones. In addition, an UPLC–Q-TOF-MS method was validated for the quantitative determination of PPAPs. The method exhibited limits of detection from 2.7 to 21.4 ng mL−1 and intra-day and inter-day variations were less than 3.7% and the recovery was in the range of 89–107% with RSD less than 9.0%. This UPLC–Q-TOF-MS method has successfully been applied to quantify 16 PPAPs in 32 samples of 10 Garcinia species, which were found to be a rich source of PPAPs. (Zhou *et al*, 2010)

Chapter Three

Methodology

3.1 Preparation of plant extract for experiments

3.1.1 Collection: *Garcinia cowa* is not so available throughout the country. The stem, bark and leaf of plant was collected from comilla, Bangladesh. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka.

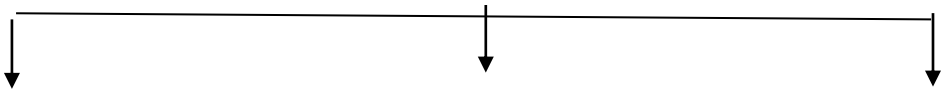
3.1.2 Process of powdering

At first the plant was cleaned to remove dust, soil etc. within them. After this the whole amount of plant was dried. The dried plants were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. The amount of powder was 550g. During powdering of sample, the blander was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blander.

3.1.3 Extraction

The fine powders of stem, bark and leaf was dissolved in three different solvents dichloromethane, methanol and petroleum ether. It thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

Garcinia cowa



Stem	**Bark**	**Leaf**
1. Petroleum ether	1. Petroleum ether	1. Petroleum ether
2. Dicholomethane	2. Dicholomethane	2. Dicholomethane
3. Methanol	3. Methanol	3. Methanol

3.1.4 Filtration

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

3.1.5 Evaporation and extract preparation

For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reuse it. For the solvents almost 70% solvents get back into liquid form. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

Figure 13: Rotary evaporator

3.2 Cytotoxicity Test: Experimental procedure

Brine Shrimp Lethality Bioassay

3.2.1Materials

- ✓ *Anemia salina* Leach. (Brine eggs), Sea salt (NaCI)
- ✓ Small tank
- ✓ Lamp to attract Shrimps
- ✓ Pipettes (5,10ml) and Micropipette (5-50nl), (10-100ul)
- ✓ Glass vials
- ✓ Magnifying glass
- ✓ Small tank with perforated dividing dam to hatch the shrimp

3.2.2 Principle

Brine Shrimp lethality bioassay (Luo *et al.,* 2000; Mclaughlin *et al.,* 1998; Meyer *et al.,* 1982) is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. The method utilizes *in vivo* lethality in a simple zoological organism (Brine nauplii) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Brine toxicity is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity (p=0.036 and kappa = 0.56). ED_{50} values for cytotoxicities are generally about one-tenth the LC_{50} values found in the Brine Shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well as 3PS (P_{388}) *(in vivo* murine leukaemia) active extracts using the Brine lethality bioassay (Alkofahi *et al,*1988; Mclaughlin *et al,* 1998; Meyer *et al,*1982). The Brine Shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less). Further more it does not require animal serum as is needed for cytotoxicities (Mclaughlin *et al.,* 1998).

3.2.3 Preparation of seawater

38 gm sea salt (without iodine) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

3.2.4 Hatching of Brine Shrimp

Artemiasalina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps are attracted to the light (phototaxis) and so nauplii free from egg shell was collected from the illuminated part of the tank. The nauplii was taken from the fish tank by a pipette and diluted in fresh clear sea water to increase visibility and 10 naupliiwas taken carefully by micropipette.

3.2.5 Preparation of test solutions with samples of experimental plants

32 mg of each of the test samples were taken and dissolved in 20ml of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 ml with sea water. Thus the concentration of the stock solution was 1600µg/ml. Then the solution was serial diluted to 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii.

Table 6: Preparation of test solution with samples of experimental plants

Concentration (µg/ml)	Extract Solution	Sea water containing 10 nauplii	Final volume
200	2.5 ml (400µg/ml)	2.5 ml	5 ml
100	2.5 ml (200µg/ml)	2.5 ml	5 ml
50	2.5 ml (100µg/ml)	2.5 ml	5 ml
25	2.5 ml (50µg/ml)	2.5 ml	5 ml

12.5	2.5 ml (25µg/ml)	2.5 ml	5 ml
6.25	2.5 ml (12.5µg/ml)	2.5 ml	5 ml
3.125	2.5 ml(6.25µg/ml)	2.5 ml	5 ml
1.562	2.5 ml (3.12 µg/ml)	2.5 ml	5 ml

3.2.6 Preparation of control group

Control groups were used in cytotoxicity study to validate the test method and ensure that the results obtained were only due to the activity of the test agent and the effects of the other possible factors were nullified. Two types of control groups were used

i) Positive control

ii) Negative control

3.2.6.1 Preparation of the positive control group

Positive control in a cytotoxicity study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study vincristine sulfate was used. As vincristine is a very cytotoxic alkaloid it was evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06 µg/ml)

3.2.6.2 Preparation of the negative control group

50 µl of DMSO was added to each of three premarked test tubes containing 4.95 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

3.2.7 Counting of nauplii

After 24 hours, the test tube were inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data,

the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The mortality was corrected using Abott's formula (*Abott W. S., 1925*)

$$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$$

Where, P_o= Observed mortality

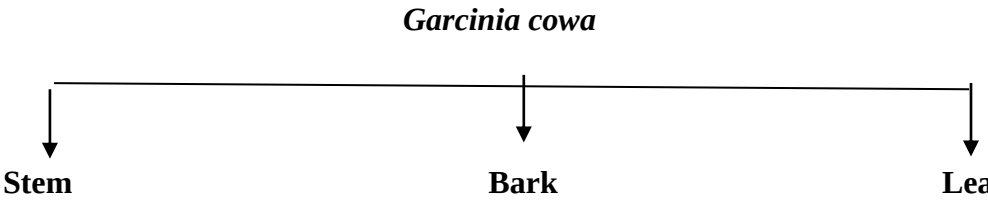
P_c= Control mortality

The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}). This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time and determined by linear regression method from plotting % mortality against correspondent log of concentration.

Chapter Four

Result and Discussion

4.1 Result of Cytotoxicity Assay of *Garcinia cowa*



1.Petroleum ether	1. Petroleum ether	1.Petroleum ether
2. Dicholomethane	2. Dicholomethane	2. Dicholomethane
3. Methanol	3. Methanol	3. Methanol

4.1.1 Stem

4.1.1.1 Cytotoxic assay of Stem (petroleum ether)

Table 7: Effect of *Garcinia cowa* stem (petroleum ether extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	50
10	100	2	50
10	50	1.69897	40
10	25	1.39794	40
10	12.5	1.09691	40
10	6.25	0.79588	30
10	3.125	0.49485	30
10	1.562	0.193681	20
10	0	0	0

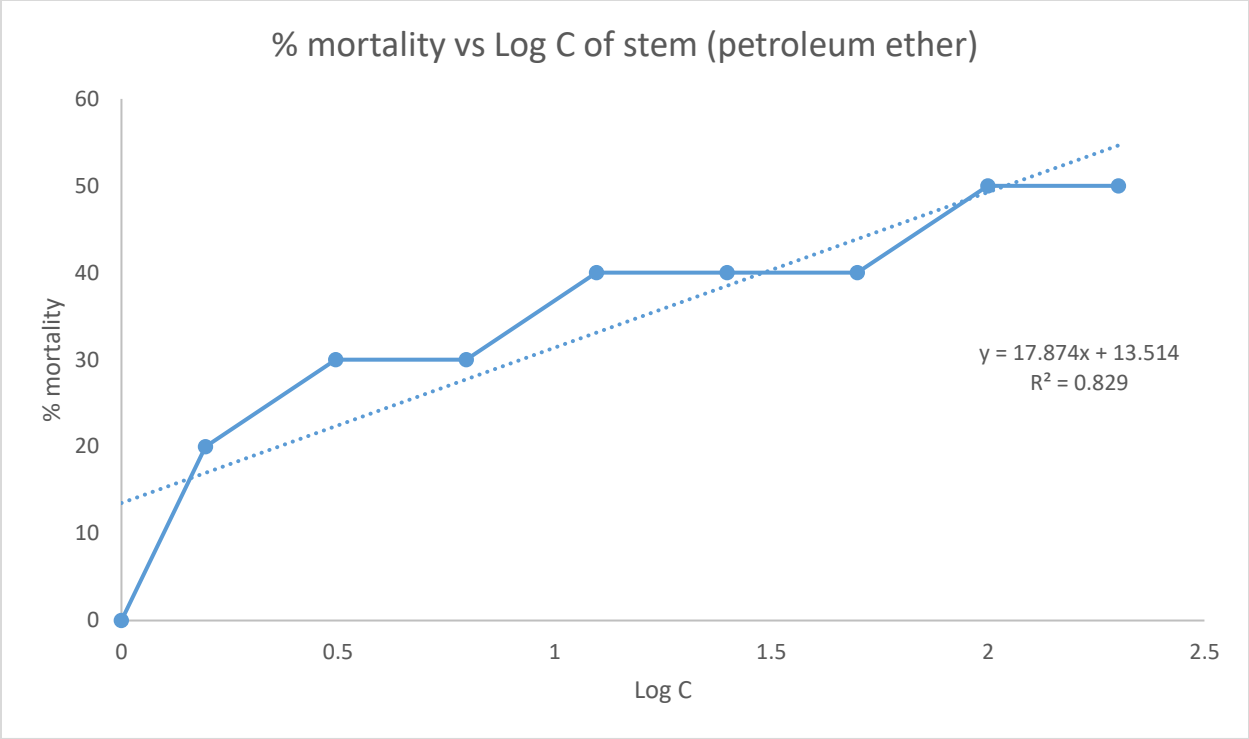


Figure 14: Effect of *Garcinia cowa* stem (petroleum ether extract) on shrimp nauplii

4.1.1.2 Cytotoxic assay of Stem (Dicholoromethane)

Table 8: Effect of *Garcinia cowa* stem (Dicholoromethane extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	70
10	100	2	70
10	50	1.69897	60
10	25	1.39794	60
10	12.5	1.09691	50
10	6.25	0.79588	50
10	3.125	0.49485	50
10	1.562	0.193681	40
10	0	0	0

Figure 15: Effect of *Garcinia cowa* stem (Dicholoromethane extract) on shrimp nauplii

4.1.1.3 Cytotoxic assay of Stem (methanol)

Table 9: Effect of *Garcinia cowa* stem (methanol extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	60
10	100	2	50
10	50	1.69897	50
10	25	1.39794	40
10	12.5	1.09691	30
10	6.25	0.79588	30
10	3.125	0.49485	20
10	1.562	0.193681	20
10	0	0	0

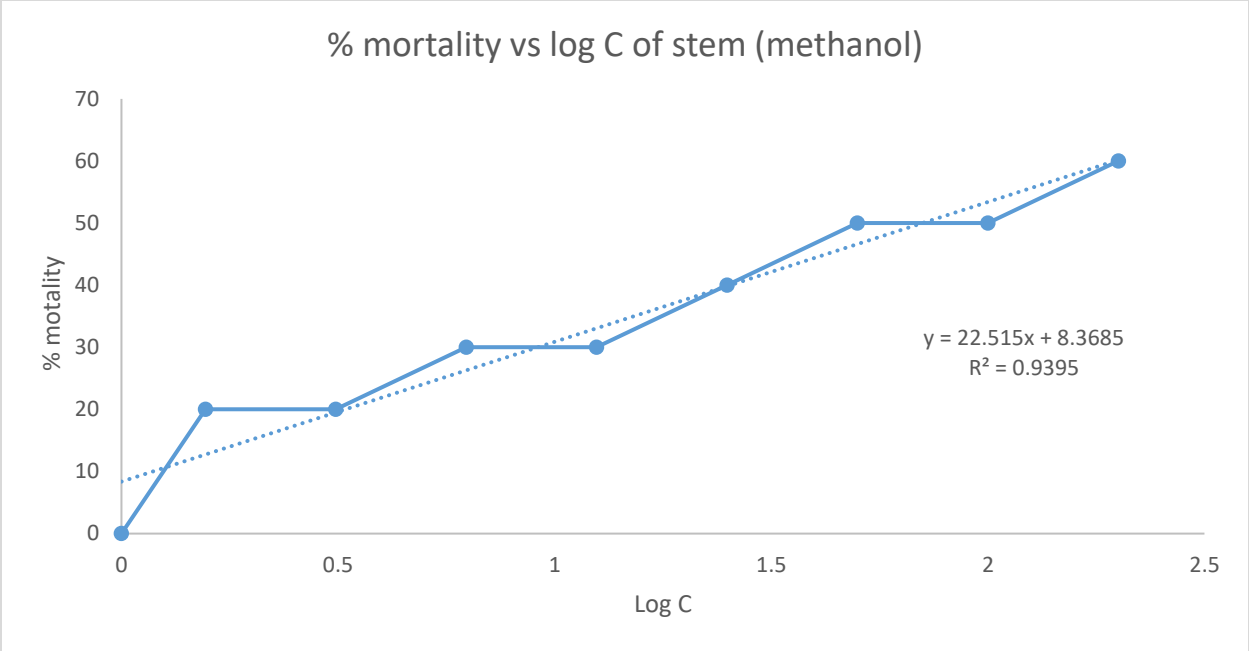


Figure 16: Effect of *Garcinia cowa* stem (methanol extract) on shrimp nauplii

4.1.2 Bark

4.1.2.1 Cytotoxic assay of Bark (petroleum ether)

Table 10: Effect of *Garcinia cowa* bark (Petroleum ether extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	60
10	100	2	50
10	50	1.69897	40
10	25	1.39794	30
10	12.5	1.09691	30
10	6.25	0.79588	20
10	3.125	0.49485	20
10	1.562	0.193681	20
10	0	0	0

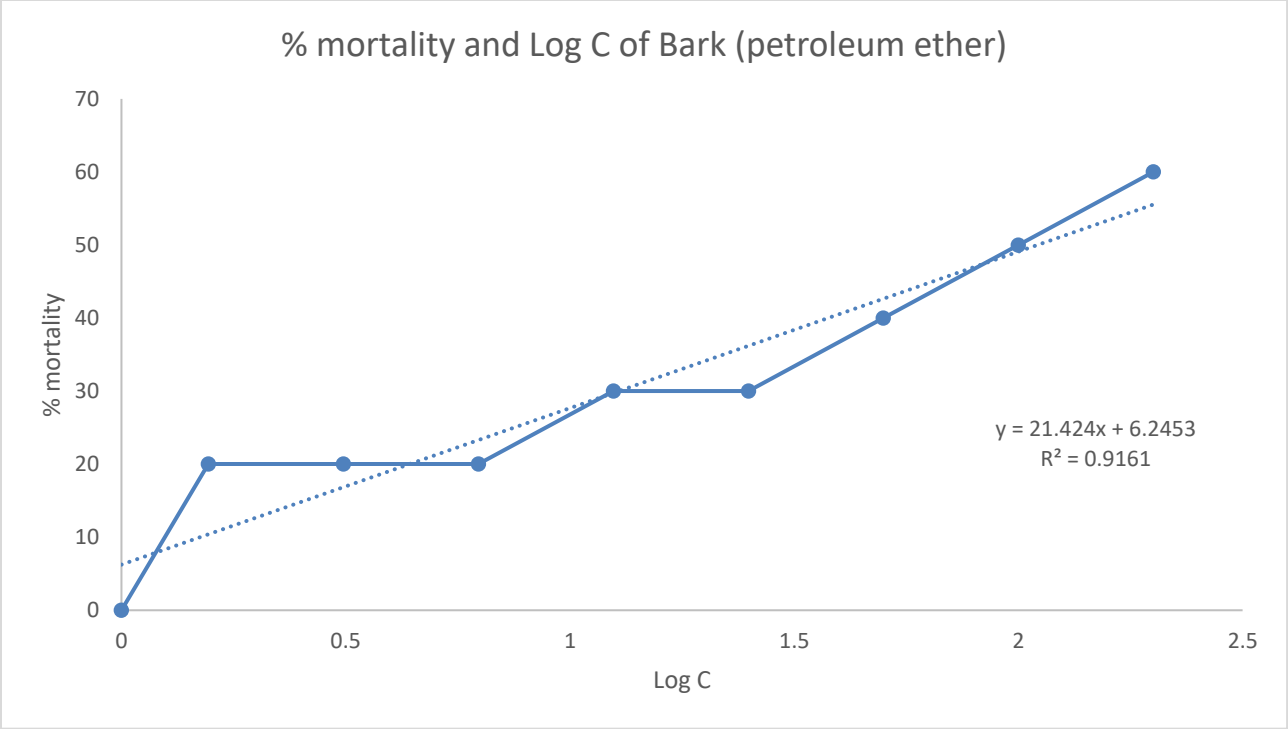


Figure 17: Effect of *Garcinia cowa* bark (Petroleum ether extract) on shrimp nauplii

4.1.2.2 Cytotoxic assay of Bark (dicholormethane)

Table 11: Effect of *Garcinia cowa* bark (Dicholoromethane extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	50
10	100	2	50
10	50	1.69897	40
10	25	1.39794	30
10	12.5	1.09691	30
10	6.25	0.79588	30
10	3.125	0.49485	20
10	1.562	0.193681	20
10	0	0	0

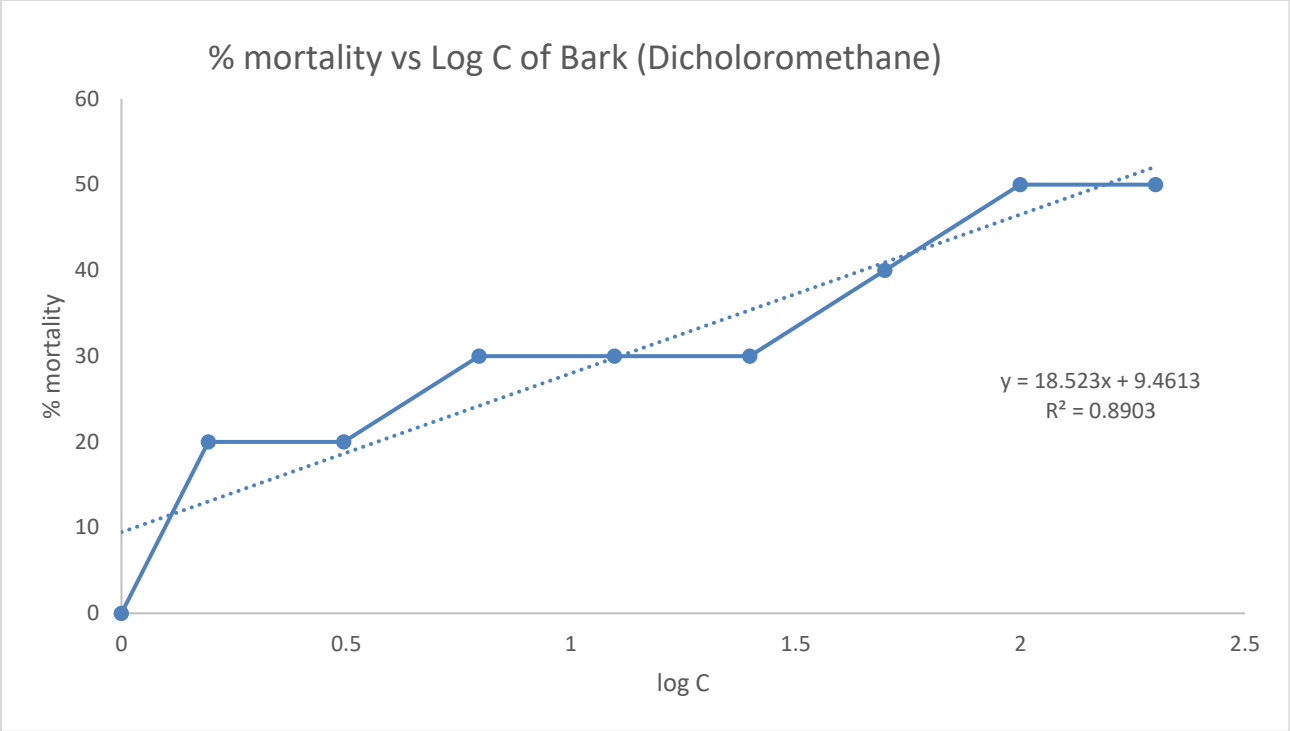


Figure 18: Effect of *Garcinia cowa* bark (Dicholoromethane extract) on shrimp nauplii

4.1.2.3 Cytotoxic assay of Bark (methanol)

Table 12: Effect of *Garcinia cowa* bark (methanol extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	70
10	100	2	70
10	50	1.69897	60
10	25	1.39794	60
10	12.5	1.09691	50
10	6.25	0.79588	50
10	3.125	0.49485	40
10	1.562	0.193681	20
10	0	0	0

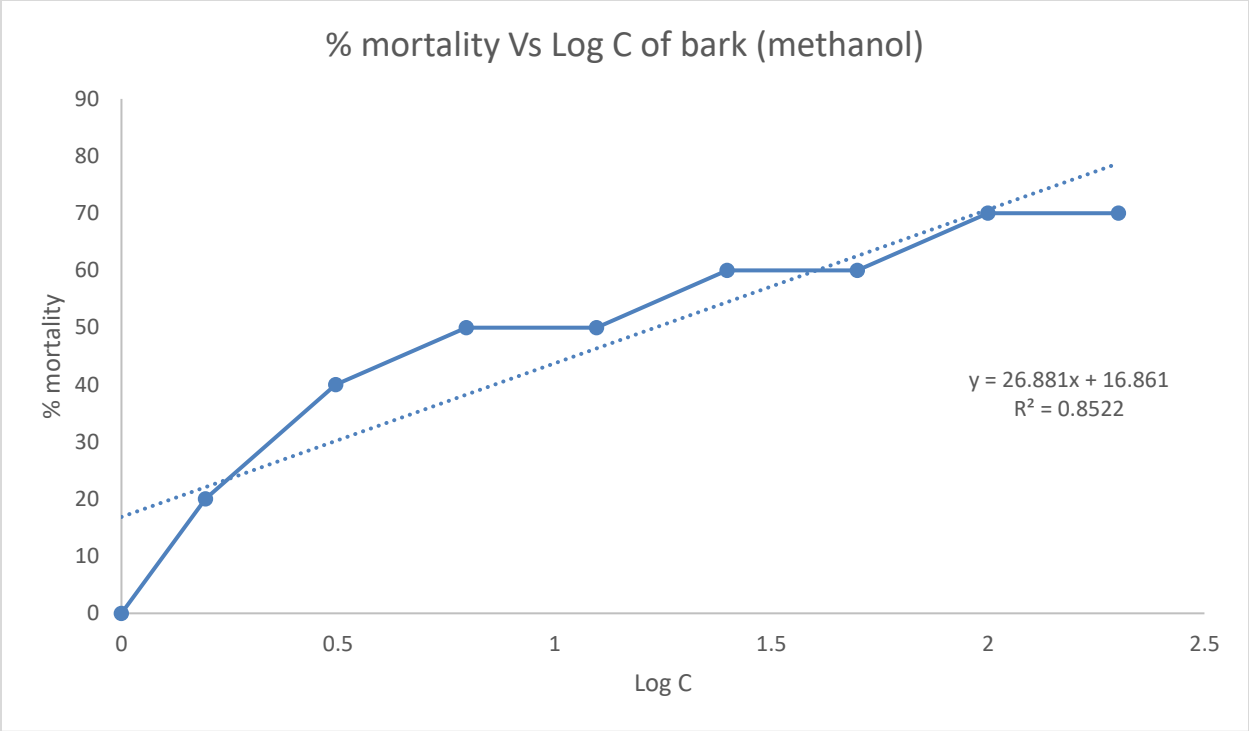


Figure 19: Effect of *Garcinia cowa* bark (methanol extract) on shrimp nauplii

4.1.3 Leaf

4.1.3.1 Cytotoxic assay of Leaf (petroleum ether)

Table 13: Effect of *Garcinia cowa* leaf (Petroleum ether extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	60
10	100	2	60
10	50	1.69897	50
10	25	1.39794	40
10	12.5	1.09691	40
10	6.25	0.79588	30
10	3.125	0.49485	30
10	1.562	0.193681	20
10	0	0	0

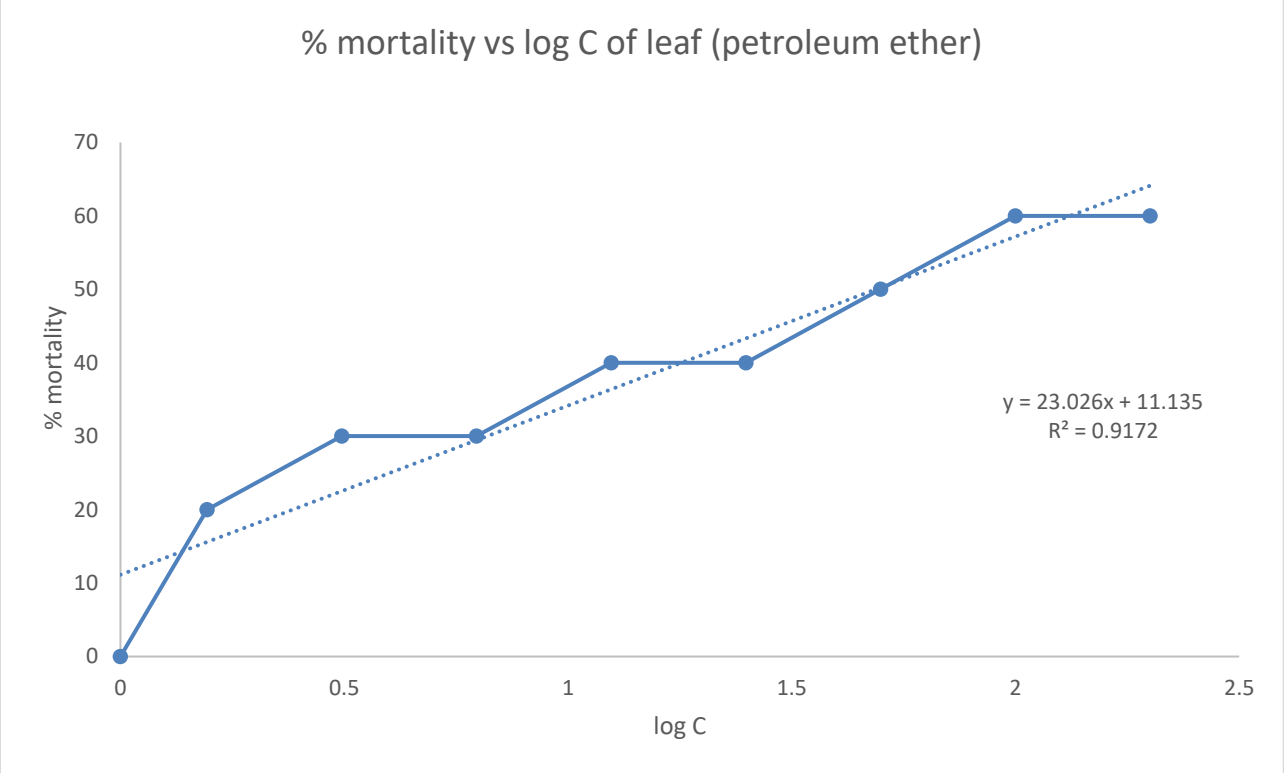


Figure 20: Effect of *Garcinia cowa* leaf (Petroleum ether extract) on shrimp nauplii

4.1.3.2 Cytotoxic assay of Leaf (dichloromethane)

Table 14: Effect of *Garcinia cowa* leaf (Dichloromethane extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	80
10	100	2	70
10	50	1.69897	70
10	25	1.39794	60
10	12.5	1.09691	50
10	6.25	0.79588	50
10	3.125	0.49485	40
10	1.562	0.193681	30
10	0	0	0

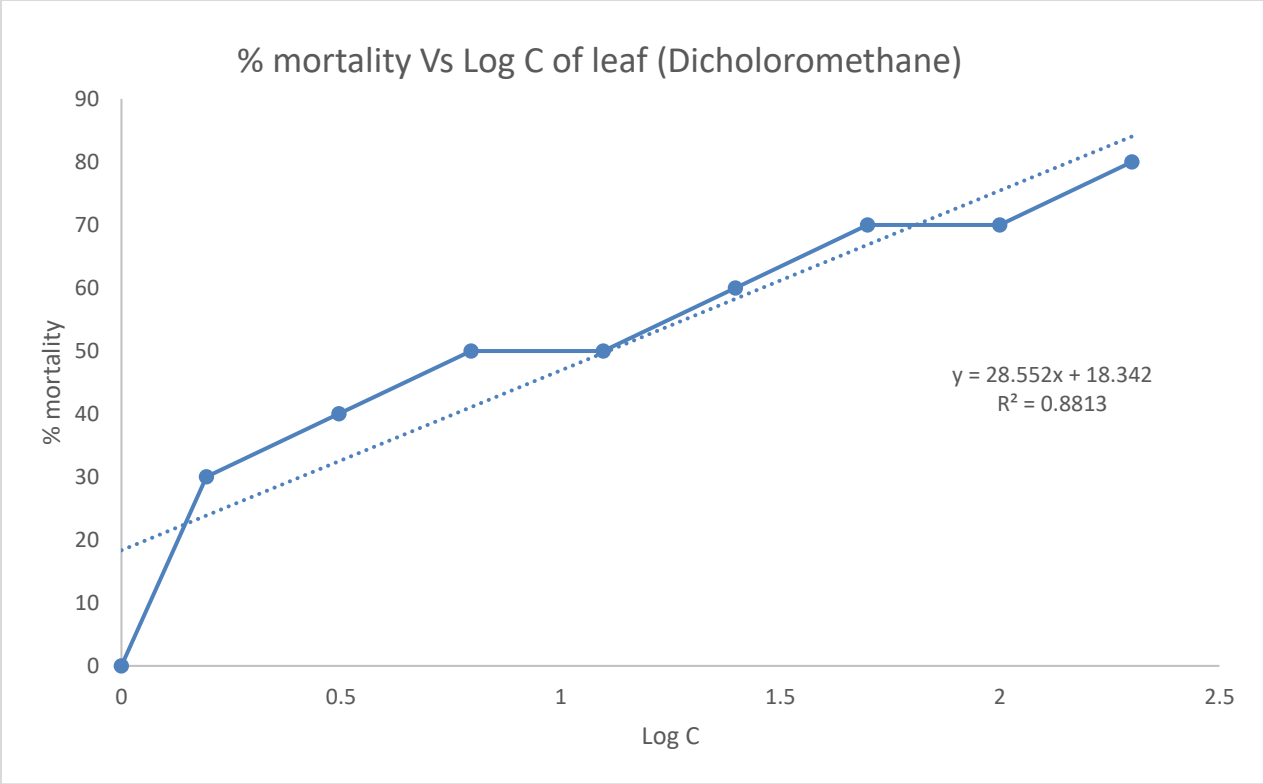


Figure 21: Effect of *Garcinia cowa* leaf (Dichloromethane extract) on shrimp nauplii

4.1.3.3 Cytotoxic assay of Leaf (methanol)

Table 15: Effect of *Garcinia cowa* leaf (Methanol extract) on shrimp nauplii

Number of nauplii taken	concentration	Log c	% mortality
10	200	2.30103	70
10	100	2	70
10	50	1.69897	60
10	25	1.39794	50
10	12.5	1.09691	30
10	6.25	0.79588	30
10	3.125	0.49485	20
10	1.562	0.193681	20
10	0	0	0

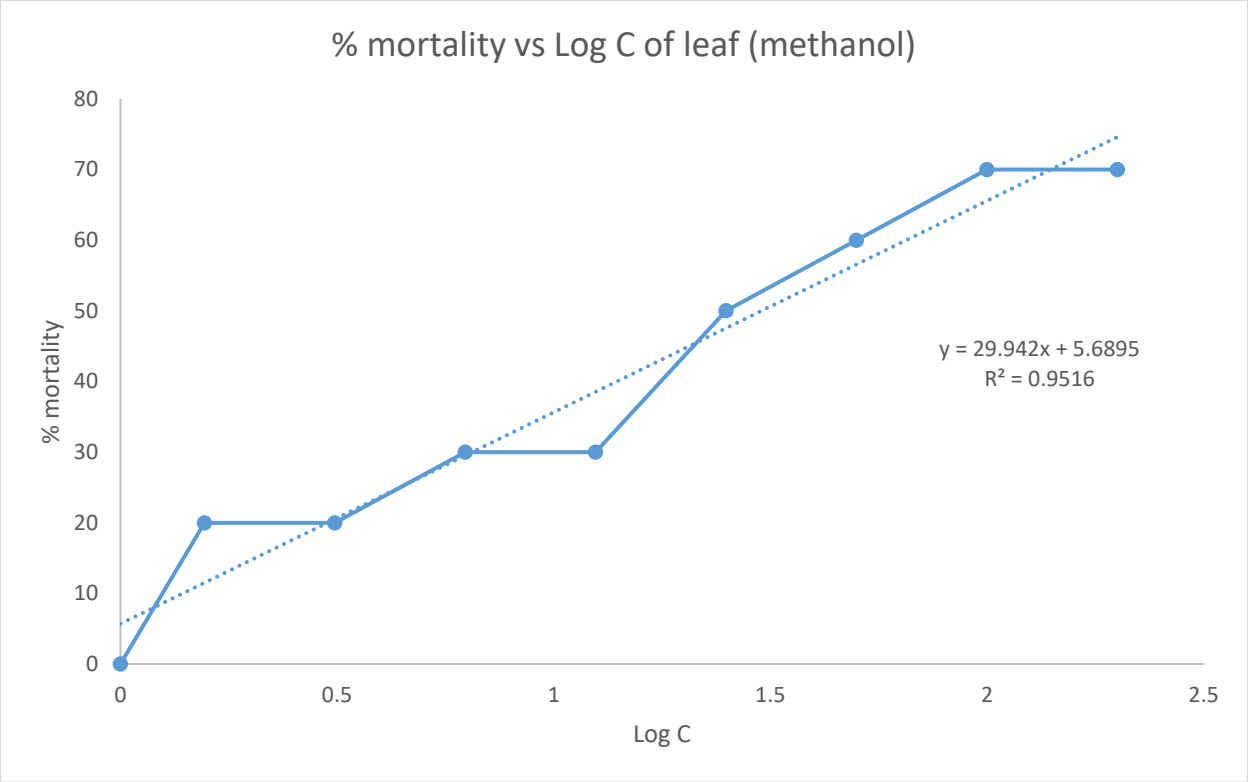


Figure 22: Effect of *Garcinia cowa* leaf (Methanol extract) on shrimp nauplii

Table 16: LC_{50} and R^2 value of different samples

Serial	Sample	Equation	Value of X (log LC_{50})	LC_{50}	R^2
1	Stem (petroleum Ether)	y= 17.874x +13.514	2.0412	109.95	0.829
2	Stem (dicholoro methane)	y=22.171x+ 25.417	1.1087	12.48	0.7086
3	Stem (methanol)	y= 22.515x+ 8.3685	1.8490	70.63	0.9395
4	Bark (petroleum ether)	y=21.424x+ 6.2453	2.0423	110.23	0.9161
5	Bark (dicholoro methane)	y=18.523x+ 9.4613	2.1885	154.34	0.8903
6	Bark (methanol)	y=26.881x+ 16.861	1.2328	17.09	0.8522
7	Leaf (petroleum	y=23.026x+ 11.135	1.6878	48.73	0.9172

	ether)				
8	Leaf (dichloromethane)	y=28.552x + 18.342	1.1088	12.84	0.8813
9	Leaf (methanol)	y=29.942x+ 5.6895	1.4799	30.19	0.9516

4.2 Discussion

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phyto medicines regards the maintenance of consistent medicinal quality in botanical medicines. Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation from *Garcinia cowa* of the family Clusiaceae tribally used in various disease conditions. In my experiment it shows very positive result for cytotoxic activity. The plant *Garcinia cowa* has been used for the general promotion of health and longevity by Asian tribal (specially Chakma, Marma and Tanchunga). It is used as a traditional medicine for the treatment of various diseases such as spasm, dysentery, headache. The ethanolic extracts of the leaf also contain antibacterial property. The aim of the present study was to evaluate the cytotoxic activity of stem, bark and leaf of *Garcinia cowa*. Due to its huge therapeutic use by the tribal I got interested to do experiment on this plant. The therapeutic value of medicinal plants lies in the various chemical constituents in it.The brine shrimp lethality bioassay was performed to evaluate the cytotoxicity activity. From this test, IC_{50} value of stem (109.95, 12.48, 70.63), bark (110.23, 154.34, 17.09) and leaf (48.73, 12.48, 30.19) were calculated for petroleum ether, dicholoromethane and methanol solvents respectively.

The R^2 value were approximately stem (0.829, 0.7086, 0.9395), bark (0.9161, 0.8903, 0.8522) and leaf (0.9172, 0.8813, 0.9516). so it is evident that stem and leaf is highly cytotoxic. The bark is mild to moderate cytotoxic. Methanol could extract the cytotoxic constituents better from each of the samples. From literature review, I found out that previous studies were done to determine the cytotoxic activity of stem and bark of *Garcinia cowa.* Both stem and barks were found to be cytotoxic. (Elidahanum *et al*, 2005), (Shen *et al*, 2007). So this result rhymes with previous findings. This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind

CHAPTER FIVE

CONCLUSION

Conclusion

From the result of my study, it can be concluded that, in case of anticancer drug preparation this plant extracts can be treated as a good anticancer drug candidate as it has notable cytotoxic effect.

With only few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. In my experiment it shows very positive result for cytotoxic activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

CHAPTER SIX

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