Phytochemical Screening, Antioxidant & Antimicrobial Investigations of Methanolic Extract of Bark of Garcinia cowa

A Dissertation Submitted to the Department of Pharmacy, East West University in the Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

Submitted by

Fahmida Akhter Trisha

ID: 2013-1-70-025

Department of Pharmacy

East West University

Declaration by the Research Candidate

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

Fahmida Akhter Trisha
ID: 2013-1-70-025
Department of Pharmacy
East West University
Aftabnagar, Dhaka

Certificate by the Supervisor

This is to certify that the dissertation, entitled '**Phytochemical Screening, Antioxidant and Antimicrobial Investigations of Methanolic Extract of bark of *Garcinia cowa***' is a research work carried outby **Fahmida Akhter Trisha** (ID: 2013-1-70-025) in 2017, under the supervisionand guidance of me, in partial fulfillment of the requirement for the degree ofBachelor of Pharmacy. The thesis has not formed the basis for the award of anyother degree/diploma/fellowship or other similar title to any candidate of anyuniversity.

Nazia Hoque
Assistant Professor

Department of Pharmacy,

East West University, Dhaka.

Endorsement by the Chairperson

This is to certify that the dissertation, entitled '**Phytochemical Screening, Antioxidant and Antimicrobial Investigations of Methanolic Extract of bark of *Garcinia cowa***' is a research work carried out by **Fahmida Akhter Trisha** (ID: 2013-1-70-025), 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.

Dr. Chowdhury Faiz Hossain
Chairperson and Professor
Department of Pharmacy
East West University
Aftabnagar, Dhaka

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Dedication

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

Abstract

The aim of this study was to determine the phytochemical screening , antioxidant activity and antibacterial activity of the methanolic extract of bark of *Garcinia cowa*. After doing the phytochemical screening , revealed the presence of flavanoid , saponin , steroid and tannin and showed the absence of alkaloids and amtioxidant activity was measured by DPPH scavenging assay having strong DPPH scavenging activity with % inhibition of 84% compared to 92% of standard ascorbic acid in addition to total phenolic content was 411.5333 _+ 19.21805 mg / g gallic acid equivalent. It has also showed strong antibacterial activity against *Shigella dysenteriae* (zone of inhibition ranged from 15 to 23 mm) and Salmonella paratyphi (zone of inhibition ranged from 13 to 18 mm) and Bacillus sereus (zone of inhibition ranged from 13 to 16 mm) and E. coli (zone of inhibition ranged from 9 to 15 mm) . This study might be helpful to further research to isolate chemical constituents responsible for different medicinal value and drug development.

Key Words: *Garcinia cowa*, flavonoid , saponin, steroid, DPPH, *Shigella dysenteriae.*

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INTRODUCTION

1.1 General Introduction

As our lifestyle is now getting techno-savvy, we are moving away from nature. While we cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lot of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives.

These herbal products are today are the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. It's time to promote them globally.

(Nhp.gov.in, 2017)

Plants:

Plant, any organism of the plant kingdom, as opposed to one of the animal kingdom or of the kingdoms Fungi, Protista, or Monera in the five-kingdom system of classification. (A more recent system, suggested by genetic sequencing studies, places plants with animals and some other forms in an overarching group, the eukarya, to distinguish them from the prokaryotic bacteria and archaea, or ancient bacteria.) A plant may be microscopic in size and simple in structure, as are certain one-celled algae, or a gigantic, many-celled

complex system, such as a tree. Plants are generally distinguished from animals in that they possess chlorophyll, are usually fixed in one place, have no nervous system or sensory organs and hence respond slowly to stimuli, and have rigid supporting cell walls containing cellulose. In addition, plants grow continually throughout life and have no maximum size or characteristic form in the adult, as do animals. In higher plants the meristem tissues in the root and stem tips, in the buds, and in the cambium are areas of active growth. Plants also differ from animals in the internal structure of the cell and in certain details of reproduction (infoplease, 2012)

1.2 Introduction to Medicinal Plant

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

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

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

developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

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

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

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

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

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

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

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

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

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

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

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

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

(Nhp.gov.in, 2017)

1.2.1 Why do plants have medicinal properties?

Plants produce many chemicals that are biologically active, not just in themselves, but also in other organisms. Some of these chemicals enhance their own survival.

Below are several examples of active plant ingredients that provide medicinal plant uses for humans.

Alkaloids: This group is comprised of a wide variety of plants that contain nitrogen-bearing molecules that make them very active. Many of these plants have been used to create well-known drugs used for medicinal purposes. One such example, vincristine, which was derived from the Madagascar periwinkle (Catharanthusroseus), is used to treat some types of cancer.

Bitters: This group is comprised of a variety of plants that are lumped together because of their very bitter taste. This bitterness causes stimulation of the salivary glands and digestive organs. As such, bitters can be used to improve appetite and strengthen the digestive system.

Cardiac Glycosides: These compounds are found in various medicinal plants (Foxglove, Lily of the Valley) and have strong direct action on the heart. Cardiac glycosides such as digitoxin,digoxin, and convallotoxin support heart strength and rates of contraction when failing.

Cyanogenic Glycosides: These glycocides are based upon cyanide, a very deadly poison, but in small doses, they can serve as a muscle relaxant. The bark of wild cherry and the leaves of elderberry (Sambucusracemosa) contain cyanogenic glycocides, which can be used to suppress and soothe dry coughs.

Flavonoids: Flavonoids are found widely throughout the plant world and they have a wide range of medicinal uses and actions. They often act as pigments giving a yellow or white color to flowers and fruits. Some flavonoids have anti-viral and anti-inflammatory properties.

Minerals: Many plants have high levels of minerals because they can draw minerals from the soil and can convert them into a form that is more easily used by the human body. Mineral content is often the key factor in a plant's effectiveness as a medicine.

Phenols: Phenols are plant compounds that are thought to be produced to protect againstinfection and herbivory by insects. They are often anti-inflammatory and antiseptic and can have anti-viral properties. Phenols vary in structure and range from salicylic acid (similar to aspirin) to complex sugar-containing phenolic acids.

Polysaccharides: Polysaccharides are found in all plants and comprised of multiple units of sugar molecules linked together. For medicinal purposes, the "sticky" polysaccharides produce mucilage or gums that are commonly found in bark, roots,

leaves, and seeds. These sticky polysaccharides are able to soak up large quantities of water and form jelly like masses that can be used to treat dry or irritated tissues such as skin and mucous membranes.

Proanthocyanins: These compounds are pigments, which give fruits and flowers red, purple, or blue hues and are closely related to tannins and flavonoids. These compounds have been documented to be valuable in protection of circulation specifically in the heart, eyes, and feet.

Saponins: This group of active compounds obtains its name from the fact that like soap, they produce lather when placed in water. There are two main forms of saponins: steroidal and triterpenoid.

Tannins: Most plants produce tannins. Tannins serve as a deterrent to herbivory by insects and grazing animals given that that they provide a harsh unpalatable flavor. Tannins are also useful in curing leather because of their tendency to contract and astringe tissues by binding with precipitating proteins.

Vitamins: Many plants contain high levels of useful vitamins. Many well-known fruits and vegetables have high levels of vitamin C and beta-carotene. Lesser-known vitamin containing plants like watercress, rose hips, and sea buckthorn have high levels of vitamins B, C, and E.

Volatile oils: Volatile oils are extracted from plants and are used to produce essential oils that play a very important role in medicinal botany. These oils are often very complex and can be comprised of 100 or more compounds. These oils have many uses. For example, tea tree oil is a strong antiseptic. (USDA, 2016)

1.3 Importance of some herbs with their medicinal values

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

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

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

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

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

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

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

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

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

Some herbs like aloe, sandalwood, turmeric, sheetrajhindi and kharekhasak are commonly used as antiseptic and are very high in their medicinal values.

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

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

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

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

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

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

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

Honey, turmeric, marshmallow and liquorice can effectively treat a fresh cut and wound. They are termed as vulnerary herbs.(Nhp.gov.in, 2017)

1.4 Medicinal Plants in Ancient Traditions

Plants have been used for medicinal purposes since time immemorial, and to this day, many of the important and familiar remedies originate in plants.

This chapter outlines the history and early traditions of medicinal plants in the Middle-east. The importance of the early "medicine-men" in ancient cultures, as collectors and healers, is emphasized. Archaeological findings in sites such as Iraq and Babylon, as well as clay tablets and ancient manuscripts from Egypt, Sumaria and Assyria, India and China reveal the immense body of knowledge that existed during these old times in history.

A special place is devoted to the Bible, as an ancient document describing the use of plants in this region during biblical times.

Since before the Common Era, great herbalists, such as Dioscorides, Hippocrates, Theophrastus and Galenius acted as scientists and therapists leaving us with prominent books, such as De MateriaMedica of Dioscorides. This priceless document provided the world with vast knowledge regarding hundreds of medicinal plants which are found in the region of the Middle-East. Most of these plants are still used at the present time for therapy and some of them are rich sources of natural compounds with medicinal properties.

It was only by the mid-fifteenth century that the influence of Dioscorides, and that of the classic herbalists, began to fade within European botany and medicine. During this period, and until our times, the European herbalists began researching plants for pure research purposes, which resulted in great scientific discoveries.

Although there is no doubting the predominance of chemical research in modern medicine, there is a notably increasing interest, within both medical circles and the general public alike, in plant-oriented folk medicine. Further research into the biochemical mechanisms of herbal medicines will enable a synthesis of traditional and modern methods of health care, to the benefit of all.

(Yaniv, 2014)

1.5 Antioxidants

The process of oxidation in the human body damages cell membranes and other structures, including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates unstable molecules called 'free radicals', which steal electrons from other molecules, causing damage to DNA and other cells. .

The body can cope with some free radicals and needs them to function effectively. However, the damage caused by an overload of free radicals over time may become irreversible and lead to certain diseases, including heart disease, liver disease and some cancers (such as oral, oesophageal, stomach and bowel cancers). Oxidation can be accelerated by stress, cigarette smoking, alcohol, sunlight, pollution and other factors.

1.5.1 Antioxidants and free radicals

Antioxidants are found in certain foods and may prevent some of the damage caused by free radicals by neutralising them. These include the nutrient antioxidants, vitamins A, C and E, and the minerals copper, zinc and selenium. .

Other dietary food compounds, such as the phytochemicals in plants, are believed to have greater antioxidant effects than vitamins or minerals. These are called the non-nutrient antioxidants and include phytochemicals, such as lycopenes in tomatoes and anthocyanins found in cranberries.

1.5.2 The effect of free radicals

Some conditions caused by free radicals include:

- deterioration of the eye lens, which contributes to blindness
- inflammation of the joints (arthritis)

- damage to nerve cells in the brain, which contributes to conditions such as Parkinson's or Alzheimer's disease
- acceleration of the ageing process
- increased risk of coronary heart disease, since free radicals encourage low-density lipoprotein (LDL) cholesterol to stick to artery walls
- certain cancers, triggered by damaged cell DNA.

1.5.3 Disease-fighting antioxidants

A diet high in antioxidants may reduce the risk of many diseases, including heart disease and certain cancers. Antioxidants scavenge free radicals from the body cells, and prevent or reduce the damage caused by oxidation. .

The protective effect of antioxidants continues to be studied around the world. For instance, men who eat plenty of the antioxidant lycopene (found in tomatoes) may be less likely than other men to develop prostate cancer. Lutein, found in spinach and corn, has been linked to a lower incidence of eye lens degeneration and associated blindness in the elderly. Flavonoids, such as the tea catechins found in green tea, are believed to contribute to the low rates of heart disease in Japan.

1.5.4 Sources of antioxidants

Plant foods are rich sources of antioxidants. They are most abundant in fruits and vegetables, as well as other foods including nuts, wholegrains and some meats, poultry and fish.

Good sources of specific antioxidants include:

1. allium sulphur compounds – leeks, onions and garlic
2. anthocyanins – eggplant, grapes and berries
3. beta-carotene – pumpkin, mangoes, apricots, carrots, spinach and parsley
4. catechins – red wine and tea
5. copper – seafood, lean meat, milk and nuts
6. cryptoxanthins – red capsicum, pumpkin and mangoes
7. flavonoids – tea, green tea, citrus fruits, red wine, onion and apples
8. indoles – cruciferous vegetables such as broccoli, cabbage and cauliflower
9. isoflavonoids – soybeans, tofu, lentils, peas and milk
10. lignans – sesame seeds, bran, whole grains and vegetables
11. lutein – green, leafy vegetables like spinach, and corn
12. lycopene – tomatoes, pink grapefruit and watermelon
13. manganese – seafood, lean meat, milk and nuts
14. polyphenols – thyme and oregano
15. selenium – seafood, offal, lean meat and whole grains
16. vitaminA – liver, sweet potatoes, carrots, milk, and egg yolks
17. vitaminC – oranges, blackcurrants, kiwifruit, mangoes, broccoli, spinach, capsicum and strawberries
18. vitaminE – vegetable oils (such as wheatgerm oil), avocados, nuts, seeds and whole grains
19. zinc – seafood, lean meat, milk and nuts
20. zoochemicals – red meat, offal and fish. Also derived from the plants that animals eat.

1.5.5 Vitamin supplements

There is increasing evidence that antioxidants are more effective when obtained from whole foods, rather than isolated from a food and presented in tablet form – and some supplements can actually increase cancer risk. For instance, vitamin A (beta-carotene) has been associated with a reduced risk of certain cancers, but an increase in others, such as lung cancer in smokers, if vitamin A is purified from foodstuffs. .

A study examining the effects of vitamin E found that it did not offer the same benefits when taken as a supplement. Also, antioxidant minerals or vitamins can act as pro-oxidants or damaging 'oxidants' if they are consumed at levels significantly above the recommended amounts for dietary intake. .

A well-balanced diet, which includes consuming antioxidants from whole foods, is best. If you insist on taking a supplement, seek supplements that contain all nutrients at the recommended levels.

1.5.6 General recommendations for antioxidants

Research is divided over whether or not antioxidant supplements offer the same health benefits as antioxidants in foods. It is recommended that people eat a wide variety of fresh fruits, vegetables, whole grains, lean meats and dairy products everyday. .

Your diet should include five daily serves of fruit and vegetables. One serve is a medium-sized piece of fruit or a half-cup of cooked vegetables. It is also thought that antioxidants

and other protective constituents from vegetables, legumes and fruit need to be consumed regularly from early life to be effective. See your doctor or dietitian for advice.

(Betterhealth.vic.gov.au, 2017)

1.6 Antibiotics from nature: traditional medicine as a source of new solutions for combating antimicrobial resistance

Nature has served as humankind's pharmacy for millennia. Indeed, self-medication with natural resources such as plants and fungi has not been restricted to human use alone, but has even been documented in various animals, ranging from insects to primates . Plants produce complex suites of compounds known as secondary metabolites, which are not necessary for their primary growth and function, but rather serve another role of enhancing likelihood of survival. Plants are sessile and thus highly dependent on the ability to produce and release these chemical signals into their environment for the purposes of communication and defence. Throughout ancient history, humans have learned to harness this chemical arsenal to serve their own needs. This is most apparent when considering human health and traditional forms of medicine.

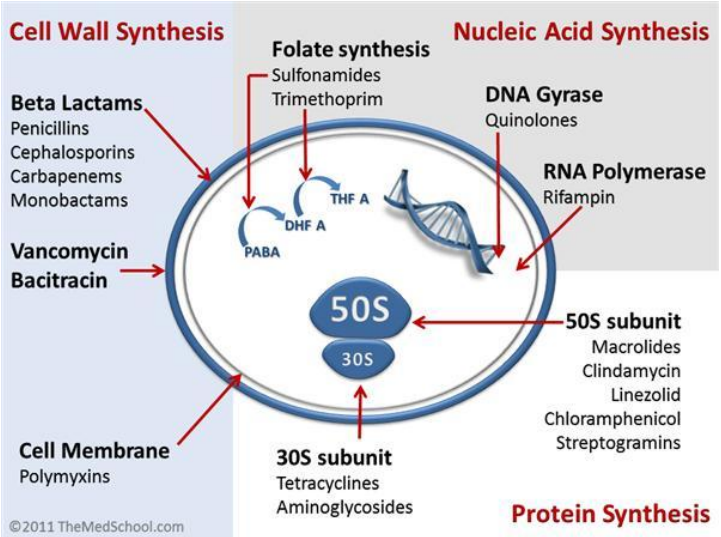


Figure 1: Mechanism of action of Antibiotics (Orthobullets.com, 2017)

1.6.1 Nature's pharmacopoeia

A number of ancient medical texts from different cultures focus heavily on the use of plant ingredients for human health. Some examples include the *Eber's Papyrus*, an ancient Egyptian scroll that dates back to 1500 BCE; Shen Nong Ben Cao, a Chinese medical text from 200 BCE; and Dioscorides' *De MateriaMedica*, which documents the Mediterranean pharmacopoeia from 50–70 CE. All include plant-based remedies for a broad number of ailments, many of which could have been attributed to infectious diseases. The tradition of using plants as medicine for the treatment and management of various infectious diseases continues even today, especially in the developing world. A 2002 WHO report noted that in Africa, up to 80% of the population uses traditional medicine (primarily plant-based) to meet their healthcare needs. Likewise, in China, traditional medicine accounts for around 40% of all healthcare delivered . The prevalence of plants in current healthcare practices should not come as a surprise, especially when one considers their predominance in ancient texts and in paleobotanical findings at archaeological sites. Many of these ancient medical practices persist in various forms of traditional medicine currently practised across the globe.

Plants are capable of producing a vast array of structurally diverse compounds, each of which serves a specific role for the plant itself (e.g., defence against phytopathogens). Sometimes, these compounds are also active against human pathogens. There are four major groups of antimicrobial compounds made by plants: phenolics and polyphenols, terpenoids and essential oils, lectins and polypeptides, and alkaloids. In most cases, bioactive plant extracts contain complex mixtures of these groups, and their combined action can yield an enhanced effect. These compounds act on bacteria via a number of mechanisms, including inactivation of proteins, adhesins and enzymes, among other targets. More recent work has revealed that certain plant compounds can also block cell-to-cell signaling pathways and quench production of virulence factors (e.g., exotoxins)

and disrupt or inhibit the formation of biofilms , which confer a protective advantage to pathogens during an infection . It is clear that we have only uncovered the tip of the iceberg in our understanding of the chemical diversity and bioactivity of plant natural products.

Under the lens of Western medicine, natural products, defined as molecular entities produced by a living organism (including mammals, plants, fungi, bacteria, etc.), and their derivatives make up roughly one-third of all FDA-approved drugs. Before the golden era of antibiotics (1950), plant natural products represented more than one-fifth (22%) of all new molecular entities used in medicine. However, since then, there has been a decline in botanical compounds used in Western medicine (8.7%). Specific to antibacterial agents, natural products and their derivatives make up 69% of all FDA-approved drugs. The majority of these come from microbes (97%), with plant products contributing just 3% to this group .

1.6.2 Benefits and challenges of plants as a source of antimicrobials

The current percentage of approved antibacterial drugs from plants, however, does not accurately reflect the potential of plant natural products for future applications as antimicrobial therapies. In part, there are some inherent difficulties in the development of plant natural products as antimicrobial pharmaceuticals:

Plant extracts are incredibly chemically complex – much more so than fungi, for example, as a single extract preparation may contain hundreds of different chemical entities. The isolation of single compounds with the desired antimicrobial bioactivity can be time consuming and requires a large amount of bulk plant material.

Rediscovery of the same compounds from different sources presents problems, and much attention must be paid to careful dereplication early in the discovery process in order to avoid time and effort spent chasing known molecular entities.

Making arrangements for access to plant specimens can sometimes be difficult, especially in an international setting. Regulations concerning plant collection permits and export/import permits differ depending on where the research is being conducted. Furthermore, as per the regulations and guidance set forth by the United Nations Convention on Biological Diversity and the Nagoya Protocol, negotiation of equitable access and benefit sharing agreements is required for such research .

Many plant-based therapies work via synergistic pathways. Synergism among compounds in a complex mixture presents unique difficulties as the scientific technology to study multiple compounds acting in unison on potentially multiple biological targets has not yet been fully developed. On the other hand, it could be argued that the synergistic activity of certain plant extracts may present a unique opportunity in the face of growing antibiotic resistance. It raises the question of whether more chemically complex formulations can outlast monotherapies by making it more difficult for microbes to evolve resistance to a multi-sided attack.

A good example of the concept of synergy comes from the traditional Chinese medicinal plant Qinghao (*Artemisia*annua L, *Asteraceae*). This species is the source of the antimalarial compound artemisinin, the discovery of which recently resulted in the 2015 Nobel Prize in Physiology or Medicine to Chinese scientist, YouyouTu . Unfortunately, the widespread emergence of resistance to artemisinin monotherapy has become increasingly problematic .Qinghao is a therapy known to have been in use for millennia, as evidenced by specific recommendations found concerning its preparation and use in an ancient text from the Jin dynasty: *The Handbook of Prescriptions for Emergency Treatments* by Ge Hong (283–343 CE). This begs the question: how is it that a traditional preparation in use for millennia did not yield resistance, yet isolation of a single compound for monotherapy resulted in widespread resistance in a short period of time?

While there are factors such as the widespread use of the drug via global distribution to consider, the topic of synergy must also be explored. Interestingly, a few recent studies have demonstrated that not only do chemically complex extracts of A. annua exhibit anti-plasmodial activity that is 6 to 18-fold greater than what was expected based on artemisinin content alone, but whole plant therapy was effective at overcoming artemisinin resistance in an animal model. Collectively, these studies support the concept that synergistic action of multiple natural products in this species are more effective and can overcome resistance noted in monotherapy models. Could this same concept hold true for the development of novel antibacterial formulations designed to overcome resistance acquisition in the future?

(Resistancecontrol.info, 2017)

1.7 Introduction to *Garcinia* genus

The genus *Garcinia* belongs to the family Clusiaceae and has been involved in ayurvedic preparations to medicate various pathophysiological disorders. The bioactive molecules like hydroxycitric acid (HCA), flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone isoforms have been isolated from the genus *Garcinia*. The genus has received the attention of pharmaceutical industries due to their immense remedial qualities. The HCA has been known for its hypolipidemic property. The polyisoprenylated benzophenone and xanthone derivatives are known for their antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, anti-ulcer, anti-protozoal, and HAT inhibiting properties. Future studies on the synthesis of therapeutically important products and their analogs and evaluation of their safety and efficacy would be of great interest. Though the genus includes more than 300 species, we have made an effort to

conceive the curative qualities of bioactive compounds of selected plants to the best of our knowledge. (Hemshekhar et al., 2011)

Figure 2 : Garcinia cowa (Source of this plant Muradnagar, Comilla)

1.8 Plant Review

Accepted scientific name : *Garcinia cowa* Roxb.

Table 1 : Synonyms of Garcinia cowa

Cambogia crassifolia Blanco	*Garcinia wallichii* Choisy
Garcinia cambogia Roxb.	*Oxycarpus gangetica* Buch.-Ham.

Garcinia cornea Roxb. ex Sm.	*Stalagmitiscowa* (Roxb.) G. Don
Garcinia lobulosa Wall.	*Stalagmitisky diana* G. Don
Garcinia roxburghii Wight	*Garcinia dioica* Sm.

Table 2 : Taxonomical Classification of Garcinia cowa

Kingdom	Plantae
Phylum	Tracheophyta
Class	Mangoliopsida
Order	Malpighiales
Family	Clusiaceae
Genus	*Garcinia*

Figure 3 : Bark of Garcinia cowa

Distribution:

peninsular Malaysia, China (Yunnan), Bangladesh, Cambodia, Sikkim, Darjeeling, Laos, Vietnam, Andamans (North Andamans, Middle Andamans, South Andamans, Little Andaman Isl.), Myanmar [Burma] (widespread), India (Bihar, West Bengal, Sikkim, Assam, Nagaland, Tripura, Meghalaya, Orissa), Thailand.

(Catalogueoflife.org, 2017)

Description:

Evergreen trees, to 15 m high, bark smooth, surface greyish-brown; blaze creamy-yellow; exudation yellow, sticky, scanty; branches horizontal; branchlets quadrangular, drooping. Leaves simple, opposite, decussate, estipulate; petiole 8-13 mm long, stout, glabrous; lamina 8-17 x 2.5-7 cm, elliptic-oblong, oblanceolate or broadly lanceolate, base acute, attenuate or cuneate, apex acute or obtuse, margin entire, glabrous, thickly coriaceous; lateral nerves 15-21 pairs, pinnate, ascending, slender, prominent, looped along the margin forming intramarginal nerve, intercostae reticulate, obscure. Flowers dioecious, small, yellow; male flowers: to 1 cm across, 3-8 in axillary or terminal fascicles; pedicels about 6 mm long; sepals 4, 4-6 mm long, unequal, broadly ovate, fleshy, yellow; petals 4, 8-10 mm long, oblong, yellow flushed with pink or red; stamens numerous on a convex fleshy receptacle; anthers oblong; filaments short; rudimentary pistil absent; female flowers: upto 1.5 cm across, 2-5 in terminal fascicles, longer than male flowers, yellow; pedicel short; staminodes in a ring of 4 bundles of 3-8 around the ovary; filaments unequal; ovary superior, subglobose, 6-8 locular, ovules one in each cell; stigma sessile, flat, deeply divided into 6-8, papillose, wedge shaped rays. Fruit a berry, 2-4 cm across, depressed, globose, with 4-8 vertical grooves, smooth, yellow, ridid, beaked; seeds 4-8, oblong with a soft aril.

Plant Growth Form :

Tree

Native Habitat :

Terrestrial (Primary Rainforest)

Preferred Climate Zone :

Tropical

Local Conservation Status :

Exotic (Horticultural / Cultivated Only)

Habitat :

Occurs in tropical evergreen forests, dry deciduous forests and sand dune forests located behind beaches.

Associated Fauna :

Gibbons like to feed on the fruits.

Ethnobotanical Uses :

Edible Plant Parts (Edible Fruits; Edible Leaves; Edible Stems)
Food (Herb & Spice : In Vietnam, the fruits are added to fish and crab soup to make it sc
The Thai use the young shoots as a flavouring.)
Medicinal (Consuming the fruits and young leaves have a laxative effect.)

Light Preference :

Full Sun, Semi-Shade

Water Preference :

Moderate Water

Propagation Method :

Seed, Stem Cutting

Mature Foliage Colour(s) :

Green

Mature Foliage Texture(s) :

Smooth

Prominent Young Flush Colour(s) :

Red

Young Flush Texture(s) :

Glossy / Shiny

Foliar Type :

Simple / Unifoliate

Foliar Shape(s) :

Non-Palm Foliage

Foliar Venation :

Pinnate / Net

Foliar Margin :

Entire

Foliar Apex / Tip :

Acute

Flower & Plant Sexuality :

Unisexual Flowers

Flower Grouping :

Cluster / Inflorescence

Flowering Habit :

Polycarpic

Figure 4: *Garcinia cowa Roxb. Fruit*

Mature Fruit Colour(s) [Angiosperms & Gymnosperms] :

Red

(Florafaunaweb.nparks.gov.sg, 2017)

Chapter 2

LITERATURE REVIEW

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

The aim of this systematic review is to examine the efficacy of *Garcinia* extract, hydroxycitric acid (HCA) as a weight reduction agent, using data from randomised clinical trials (RCTs). Electronic and nonelectronic searches were conducted to identify relevant articles, with no restrictions in language or time. Two independent reviewers extracted the data and assessed the methodological quality of included studies. Twenty-three eligible trials were identified and twelve were included. Nine trials provided data suitable for statistical pooling. The meta-analysis revealed a small, statistically significant difference in weight loss favouring HCA over placebo (MD: -0.88 kg; 95% CI: -1.75, -0.00). Gastrointestinal adverse events were twice as common in the HCA group compared with placebo in one included study. It is concluded that the RCTs suggest that Garcinia extracts/HCA can cause short-term weight loss. The magnitude of the effect is small, and the clinical relevance is uncertain. Future trials should be more rigorous and better reported.

(Onakpoya et al., 2010)

2.2 Xanthons of *Garcinia cowa*

Five xanthones have been isolated from *Garcinia cowa*Roxb. (Guttiferae):

1. cowanin
2. cowanol
3. cowaxanthone
4. 1,3,6-trihydroxy-7-methoxy-2,5-bis(3 -methyl-2-butenyl)xanthone and
5. norcowanin

Xanthones 2 and 3 showed moderate antimicrobial activity against *Staphylococcus aureus*. (naPattalung et al., 1994)

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

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

(Pillai et al., 2012)

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

Organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa* (*G. cowa*) were determined by high-performance liquid chromatography. Fresh leaves, fruits, and dried rinds were extracted with water at 120 °C for 20−30 min under 15 lbs/in^2 pressure. Also, dried rinds were extracted with solvents (acetone and methanol) using a Soxhlet extractor at 60 °C for 8 h each. The samples were injected to HPLC under gradient elution with 0.01 M phosphoric acid and methanol with a flow rate of 0.7 mL/min using UV detection at 210 nm. The major organic acid was found to be (−)-hydroxycitric acid present in leaves, fruits, and rinds to the extent of 1.7, 2.3, and 12.7%, respectively. (−)-Hydroxycitric acid lactone, and oxalic and citric acids are present in leaves, fruits, and rinds in minor quantities. This is the first report on the composition of organic acids from *G. cowa.*(Jena, Jayaprakasha and Sakariah, 2002)

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

Compounds from the leaves of methanol extract of *Garcinia cowa* was isolated and their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines was evaluated. 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)

2.6 Effects of *Garcinia cambogia* extract on serum sex hormones in overweight subjects

(-) Hydroxycitric acid (HCA), an active ingredient extracted from the *Garcinia cambogia* fruit rind, has been commonly used as a dietary supplement for weight management. Given the controversy over HCA related testicular toxicity in animal studies, we investigated changes in serum sex hormones levels as an extension of our previous double-blind placebo-controlled trial in human subjects, in which 44 participants received either *G. cambogia* extract (1667.3 mg/day equivalent to 1000 mg HCA/day) or placebo for 12 weeks. Compared to the placebo group, administration of the extract did not significantly alter the serum testosterone, estrone, and estradiol levels. Similarly, hematology, serum triacylglycerol and serum clinical pathology parameters did not reveal any significant adverse effects. The results of this preliminary investigation indicate that ingestion of *G. cambogia* extract at dose levels commonly recommended for human use does not affect serum sex hormone levels and blood parameters.(Hayamizu et al., 2008)

2.7 Evaluation of the safety and efficacy of hydroxycitric acid or *Garcinia cambogia* extracts in humans

Several studies have shown that *Garcinia cambogia* plays an important role in the regulation of endogenous lipid biosynthesis. This effect is specially attributed to (-)-hydroxycitric acid (HCA) inhibiting the enzyme ATP-dependent citrate lyase, which catalyzes the cleavage of citrate to oxaloacetate and acetyl-CoA. Although several studies have found that the administration of *G. cambogia* extracts is associated with body weight and fat loss in both experimental animals and humans, we should be cautious when interpreting the results as other randomized, placebo-controlled clinical trials have not reported the same outcomes. Furthermore, most studies in humans have been conducted on small samples and mainly in the short term. None of them have shown whether these effects persist beyond 12 weeks of intervention. Therefore, there is still little evidence to support the potential effectiveness and long-term benefits of *G.*

cambogia extracts. With regard to toxicity and safety, it is important to note that except in rare cases, studies conducted in experimental animals have not reported increased mortality or significant toxicity. Furthermore, at the doses usually administered, no differences have been reported in terms of side effects or adverse events (those studied) in humans between individuals treated with *G. cambogia* and controls.(Márquez et al., 2012)

2.8 Diuretic activity of leaves of *Garcinia cambogia* in rats

The present study was undertaken to establish the diuretic activity of ethanol and aqueous extract of dried leaves of *Garcinia cambogia* in rats. Aqueous and ethanol extracts of leaves were administered to experimental rats orally at doses of 100 and 200 mg/kg and compared with furosemide (20 mg/kg, intraperitoneally) as the standard. The parameters measured for diuretic activity were total urine volume, urine concentration electrolytes such as sodium, potassium and chloride have been evaluated . The rats treated with ethanol extract of *Garcinia cambogia* and aqueous extract of *Garcinia cambogia* in a dose of 100 and 200 mg/kg showed higher urine output when compared to the respective control. Both ethanol and aqueous extracts have showed a significant dose-dependent increase in the excretion of electrolytes when compared to the control group.(Mathew et al., 2011)

2.9 Efficacy of *Garcinia Cambogia* on Body Weight, Inflammation and Glucose Tolerance in High Fat Fed Male Wistar Rats

Obesity leads to derangements in lipid and glucose homeostasis resulting in various metabolic complications. Plants containing vital phytochemicals are known to possesanti obesity properties and have proved to exert beneficial effects in obesity.

The present study was aimed to investigate the effects of *Garcinia Cambogia* on body weight, glucose tolerance and inflammation in high fat diet fed male Wistar rats.

Five month old male wistar rats (n=40) were divided into four groups. Two groups were fed with standard rodent diet and the remaining two with 30% high fat diet. One group in each of the two sets received the crude ethanolic extract of *Garcinia Cambogia* at a dose of 400mg/kg body weight/day for ten weeks. Body weight, intraperitoneal glucose tolerance test, leptin, tumour necrosis factor-α (TNF-α) and renal function (urea, creatinine, uric acid) were studied.

High fat diet fed rats showed increased body weight gain, glucose intolerance, elevated levels of plasma leptin and TNF-α. Supplementation of *Garcinia Cambogia* extract (GE) along with high fat diet significantly decreased body weight gain, glucose intolerance, plasma leptin and TNF-α level. No significant changes were observed in the renal function parameters in any of the groups.

Supplementation of the *Garcinia Cambogia* extract with high fat diet reduced body weight gain, inflammation and glucose intolerance.

(Sripradha, 2015)

2.10 Acute liver failure associated with *Garcinia cambogia* use

Millions of Americans regularly use herbal supplements, but many are unaware of the potential hidden dangers. Numerous supplements have been associated with hepato toxicity and, indeed dietary/herbal supplements represent an increasingly common source of acute liver injury. We report a case of acute liver failure requiring liver transplantation associated with the use of *Garcinia cambogia*, a supplement widely promoted for weight loss. When patients present with acute hepatitis or liver failure from an unknown etiology, a careful history of supplement use should be performed.(Corey et al., 2015)

2.11 In vitro chromosome aberration test and In vivo micronucleus test of Ca-type *Garcinia* extract

The induction of chromosome aberration of Ca-type *Garcinia cambogia* extract containing about 65% (-)-hydroxycitric acid was investigated by use of the chromosome aberration test in cultured Chinese hamster lung cells (CHL/IU) and the micronucleus test in mice. In the chromosome aberration test, Ca-type *Garcinia cambogia* extract did not increase the number of cells with structural aberration and/or numerical aberrations. The micronucleus test was carried out with bone marrow cells of Slc :ddY male mice after single oral administration of up to 2,000 microg/kg. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes. These results indicate that Ca-type *Garcinia cambogia* extract does not induce chromosome aberration. (ONO et al., 2006)

2.12 *Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation

By investigating long-term effects of Garcinia Cambogia (GC), weight-loss supplement, on adiposity and non-alcoholic fatty liver disease in obese mice,

The observation was that there were no significant changes in body weight and food intake between the groups. However, the supplementation of GC significantly lowered visceral fat accumulation and adipocyte size via inhibition of fatty acid synthase activity and its mRNA expression in visceral adipose tissue, along with enhanced enzymatic activity and gene expression involved in adipose fatty acid β-oxidation. Moreover, GC supplementation resulted in significant reductions in glucose intolerance and the plasma resistin level in the HFD-fed mice. However, we first demonstrated that it increased hepatic collagen accumulation, lipid peroxidation and mRNA levels of genes related to oxidative stress (superoxide dismutase and glutathione peroxidase) and inflammatory responses (tumor necrosis factor-α and monocyte chemoattractant protein-1) as well as

plasma alanine transaminase and aspartate transaminase levels, although HFD-induced hepatic steatosis was not altered.

GC protects against HFD-induced obesity by modulating adipose fatty acid synthesis and β-oxidation but induces hepatic fibrosis, inflammation and oxidative stress.

(Kim, 2013)

Chapter 3

MATERIALS & METHODS

3.1Theory of Phytochemical Screening

3.1.1.1. Materials (Reagents and Tools) Used

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

3.1.1.2 Test Compounds

Methanol, Chloroform & Pet Ether extract of barks and leaves of *S. chelonoides.*

3.1.1.3 Preparation of Sample Solution

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

3.1.1.4 Phytochemical Tests

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

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

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

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

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

v. *Tests for alkaloid:* A small volume of each extract was neutralized by adding 1 or 2 drops of dilute H_2SO_4. This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation was observed:
   a) *Mayer's reagent:* Formation of white and cream color precipitate indicated the presence of alkaloids.
   b) *Hager's reagent:* Formation of yellow crystalline precipitate indicated the presence of alkaloids.
   c) *Wagner's reagent:* Formation of brownish-black ppt indicated the presence of alkaloids.
   d) *Dragendroff's reagent:* Formation of orange or orange-red precipitate indicated the presence of alkaloids.

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

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

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

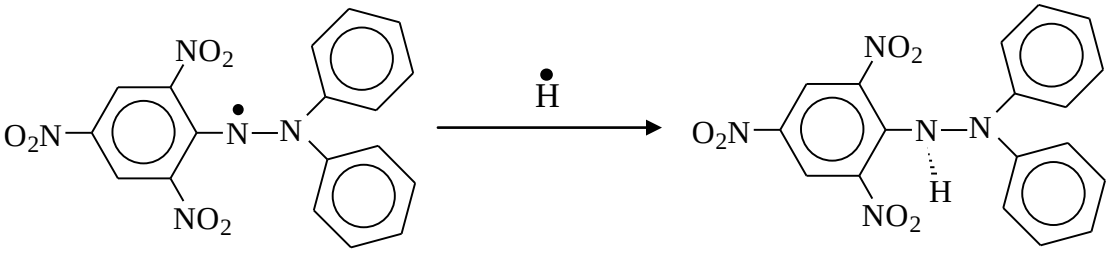
ix. *Test for tannins:* About 0.5 ml of extract was stirred with 10 ml of distilled water. Production of a blue, blue-black, green or blue-green coloration or precipitation on the addition of $FeCl_3$ (5%) reagent was taken as evidence for the presence of tannins.

3.2 Determination of Antioxidant property

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

Principle

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



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

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

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

Preparation of Standard/ Extract solution

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

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

Procedure

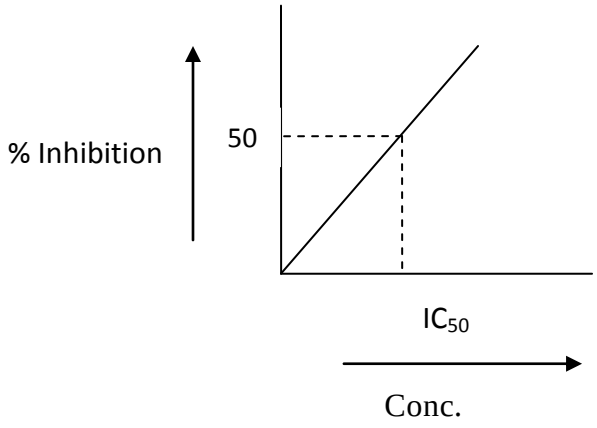
- The stock solution is serially diluted to achieve the concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml
- Each test tube contains 1ml of each concentration and is properly marked
- 2 ml of 0.004% DPPH solution in the solvent is added to each test tube to make the final volume 3 ml (caution: DPPH is light sensitive, so making the solution and adding it to the test tubes should be done in minimum light exposure)

- Incubate the mixture in room temperature for 30 minutes in a dark place
- Then the absorbance is measured at 517 nm against dilute extract solution in the solvent

Calculation

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

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



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

Principle

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

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

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

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

Preparation of 7.5% Sodium carbonate solution

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

Preparation of Standard solution

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

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

Preparation of Extract solution

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

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

Experimental Procedure

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

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

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

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

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

3.4 Antimicrobial Screening

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

3.4.1 Materials

Microorganisms

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

Test Organisms

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

Table 3: List of Bacteria Used

Gram positive Bacteria	*Gram negative Bacteria*
Bacillus cereus	*Escherichia coli*
Bacillus subtilis	*Salmonella typhi*
Staphylococcus aureus	*Pseudomonas aeruginosa*
Bacillus megaterium	*Salmonella paratyphi*
	Shigella dysenteriae
	Vibrio parahemolyticus

Culture Media and Chemicals

- Nutrient agar media
- Ethanol
- Chloroform

Equipments

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

Test Materials

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

3.3.2 Methods

Culture Preparation

Composition of culture media

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

Table 4: Composition of Nutrient agar media (1000 ml)

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

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

Sterilization Procedure:

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

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

Figure 5: Laminar Hood

Figure 6: Autoclave Machine

Preparation of Subculture

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

Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in

an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media

Preparation of Discs

Standard discs

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

Blank discs

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

Preparation of sample discs with test samples

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

3.3.3 Placement of Disc and Incubation

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

Figure 7: Incubator

3.3.4 Determination of Zone of Inhibition

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

Figure 8: Zone of inhibition

Chapter – 4

RESULT

4.0 Amount of Extract obtained from the bark of *Garcinia cowa*

The total amount of bark powder of *Garcinia cowa* taken was 718.4326 g

After petroleum ether and dichloro methane fraction, amount of extract of bark in methanol was 26.4392 g

4.1: Result of phytochemical screening:

Table 5: Result of phytochemical screening

Chemical Constituents	*Result*
Carbohydrate	-
Glycoside	-
Alkaloid	-
Saponin	+
Flavanoid	+
Steroid	+
Tannin	+

4.2: Result of Antioxidant Property

4.2.1:DPPH Free Radical Scavenging Assay

DPPH Test

Absorbance at 517 nm

Absorbance of Blank = 0.860

Table 6: Reducing power of Methanolic extract of bark of G. cowa

Concentration (µg/ml)	*Absorbance of Methanol extract of bark of G. cowa*	*% Inhibition by Methanol extract of bark of G. cowa*
0	0	0
12.5	0.487	43.37209
25	0.164	80.93023
50	0.143	83.37209
100	0.139	83.83721
200	0.132	84.65116

Table 7 : Reducing power of Ascorbic acid

Concentration (µg/ml)	*% Inhibition by Ascorbic acid*
0	0
12.5	70.66349
25	73.25201
50	80.74978
100	88.18804
200	92.7105

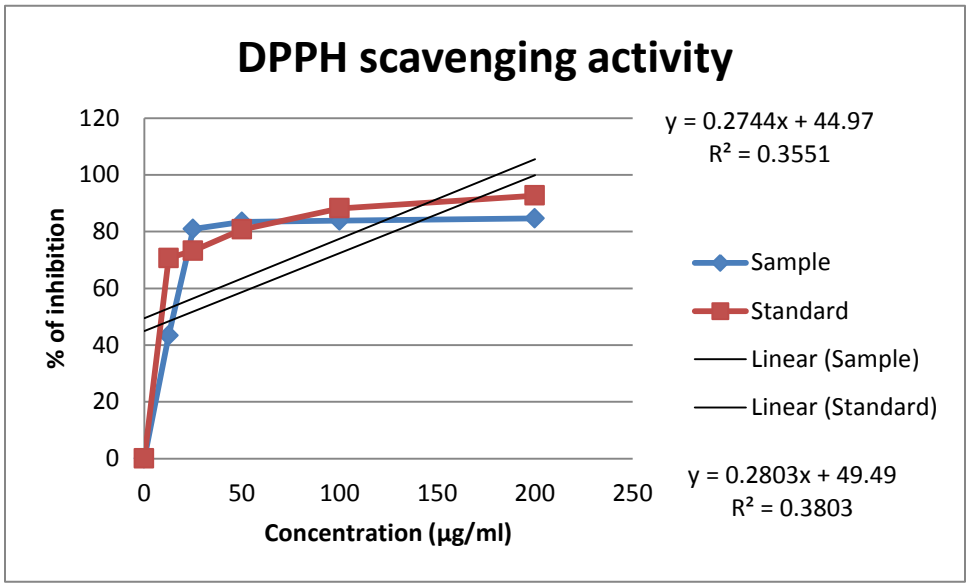


Figure 9: DPPH scavenging activity of ascorbic acid (standard) and methanolic extract of bark of Garcinia cowa

IC_{50} of extract = 18.35

IC_{50} of ascorbic acid = 1.82

4.2.2: Result of total phenolic content of the methanol extract of bark of *Garcinia cowa*

Absorbance at 765 nm

Table 8: Result of total phenolic content of the methanolic extract of bark of Garcinia cowa

Test tube no	*Absorbance*
1	394.2
2	408.2
3	432.2

Mean = 411.5333

Standard deviation = 19.21805

Total phenolic content is 411.5333 _+ 19.21805 mg / g gallic acid equivalent.

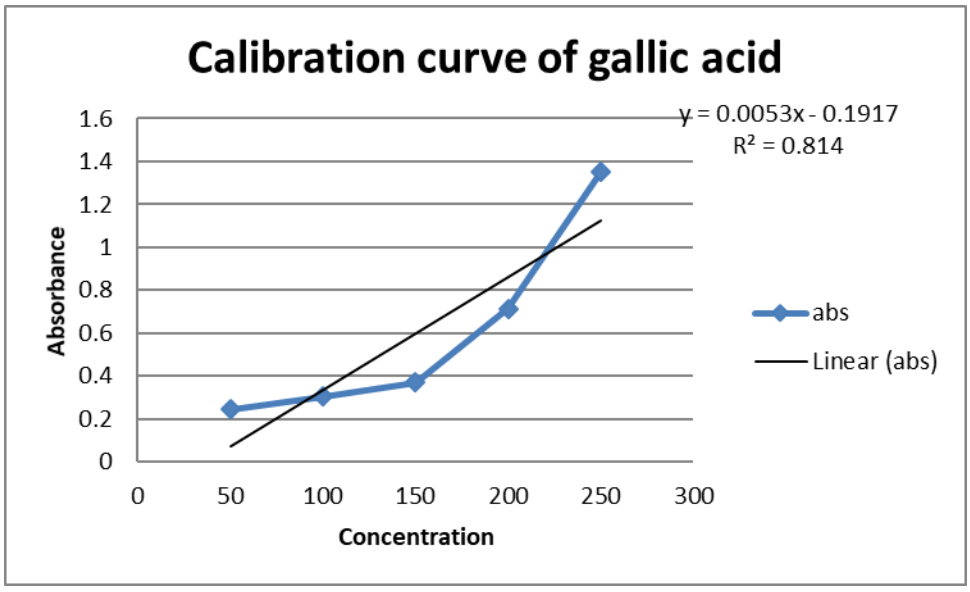


Figure 10 : Calibration curve of gallic acid

4.3: Antimicrobial Screening of the methanolic extract of bark of *Garcinia cowa*

Table 9: Zone of inhibition by methanolic extract of bark of Garcinia cowa with respect to standard Kanamycin 30µg disc

Strain	Zone of inhibition by methanolic extract of bark of G. cowa 400 µg/ disc	Zone of inhibition by methanolic extract of bark of G. cowa 800 µg/ disc	Zone of inhibition by Kanamycin 30 µg/disc
Bacillus sereus	13	16	40
Bacillus megaterium	-	-	30
Bacillus subtilis	-	-	40
Salmonella paratyphi	13	18	45
Salmonella typhi	-	-	44
Vibrio parahemolyticus	7	8	45
Staphylococcus aureaus	-	-	45
E. coli	9	15	48
Shigella dysenteriae	15	23	48
Pseudomonusaureaus	-	9	40

Chapter – 5

DISCUSSION

5.1:Discussion

5.1.1 Phytochemical screening of the methanolic extract of bark of *Garcinia cowa* :

In this study methanolic extract of the bark of *Garcinia cowa* showed positive result in the tests of Flavanoid , Saponin and Steroid .

5.1.2 DPPH Free Radical Scavenging Assay of the methanolic extract of bark of *Garcinia cowa* :

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

The reducing power of methanolic extract of bark of *Garcinia cowa* was determined by comparing it with the standard ascorbic acid using the 1,.1-diphenyl-2-picrylhydrazyl (DPPH) reduction method. The present result suggest that the tested plant extract has potent antioxidant property. It has % inhibition of 84% compared to 92% of this by standard. Since a variety of constituents is present in the extract studied. Reducing power of extract was very potent and the power of extract was increased with quantity of sample. The plant extract could reduce the most DPPH, which had a lesser reductive activity than the standard of Ascorbic acid. It becomes difficult to describe all properties selectively to any one group of constituents without further studies , which are beyond the scope of this paper. So , further extensive investigations are necessary to find out the active principles present in this plants.

5.1.3 Total phenolic content of the methanolic extract of bark of *Garcinia cowa* :

The content of total phenolic compounds of plant extracts was determined as described previously (Velioglu*et al.,* 1998) using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton*et al.,* 1999). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Vinson *et al.,* 2005).Total phenolic content is 411.5333 _+ 19.21805 mg / g gallic acid equivalent.

5.1.4 Antimicrobial Assay of the methanolic extract of bark of *Garcinia cowa* :

The Antimicrobial Activity of the methanol extract of bark of *Garcinia cowa* was tested against 4 Gram positive bacteria and 6 Gram negative bacteria. The highest antimicrobial activity was shown against *Shigella dysenteriae*, the diameter of zone of inhibition was 23 mm compared to the 48 mm of diameter of zone of inhibition of the standard Kanamycin 30 μg/disc.In case of *Bacillus sereus* the diameter of zone of inhibition was 16 mm compared to the 40 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc. In case of *Salmonella paratyphi* the diameter of zone of inhibition was 18 mm compared to the 80 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc. In case of *Vibrio parahemolyticus* the diameter of zone of inhibition was 8 mm compared to the 45 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc.. In case of *E. coli* the diameter of zone of inhibition was 15 mm compared to the 48 mm of diameter of zone of inhibition of the standard Kanamycin 30 µg/disc.

Chapter - 6

CONCLUSION

6.1: Conclusion

From the result of this study , it can be concluded that , using in vitro experiments established that methanol extract of *Garcinia cowa* nhibits the bacterial growth . In case of anticancer drug preparation this plant extract may be treated as a good candidate as it has notable cytotoxic effect. In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect .

From the result of DPPH scavenging assay and Phenolic content , it can be said that it has very good activity as antioxidant and might be used as anti-inflammatory or anti-viral agent also which may be the scope of the further study.

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

Chapter – 7

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