Pharmacological Investigation of Leaves of *Carica papaya*

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BECHELOR IN PHARMACEUTICAL SCIENCES

SUBMITTED BY
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CERTIFICATE

This is to certify that, the research work on "Pharmacological Investigation of Leaves of "***Carica papaya***" submitted to the department of pharmacy, East West University, Aftabnagor, Dhaka, in partial fulfillment of the requirement for the degree of bachelor of pharmacy (B.Pharm) was carried out by Ruhama Chowdhury, ID# 2009-3-70-043 under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the resources of the information in this connection are duly acknowledged.

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This is to certify that, the research work on "Pharmacological Investigation of Leaves of "***Carica papaya***" submitted to the department of pharmacy, East West University, Aftabnagor, Dhaka, in partial fulfillment of the requirement for the degree of bachelor of pharmacy (B.Pharm) was carried out by Ruhama Chowdhury, ID# 2009-3-70-043 under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the resources of the information in this connection are duly acknowledged**.**

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Abstract

The study was designed for pharmacological investigation of methanolic extract of leaves of ***Carica papaya*** and screening of their biological activities like, antimicrobial and free antioxidant activities. The powdered leaves of ***Carica papaya*** was extracted with methanol. This study was conducted using only the methanolic extrarct.

In antimicrobial activity investigation, methanolic crude extract of *Carica papaya* (leaves) showed moderate activity against the tested bacteria. They were appeared very little zone of inhibition against gram positive & gram negative bacteria. The three different concentration of methanolic extract of papaya leaves was used in antibacterial test. Which is compared to kanamycin (30 µg/disc) used as positive control in this study. The range of zone of inhibition was observed in between 6-11 mm depending on the concentration of the extract.

Another study was conducted to evalute the total antioxidant capacity of methanolic extract of *carica papaya* leaves using 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) free radical scavenging method. In evaluation of free radical scavenging activity it was found that crude methanolic extract of *carica papaya* (leaves) exhibited moderate free radical scavenging activity.

This thesis paper is dedicated to Almighty Allah

Chapter-1
Introduction

1.1 Introduction

The study of disease and their treatment have been existing since the beginning of human civilization. Norman R. Farnsworth of the University of Illinois declared that, for every disease that affect mankind there is a treatment and cure occurring naturally on the earth. Plant kingdom is one of the major search areas for effective works of recent days. The importance of plants in search of new drugs is increasing with the advancements of medical sciences. For example, ricin, a toxin produced by the beans of *Ricinus communis*, has been found to be effectively couple to tumor targeted monoclonal antibiotics and has proved to be a very potent antitumor drug. Further have the HIV inhibitory activity has been observed in some novel coumarins (complex angular pyranocoumarins) isolated from *Calophyllum lanigerum* and glycerrhizin from Glycerrhiza species.

Ayurvedic medicine is a system of healing that relies heavily on herbs and other plants including oils and common spices. Currently, more than 600 herbal formulas and 250 single plant drugs are included in the "pharmacy" of Ayurvedic treatments. Historically, Ayurvedic medicine has grouped plant compounds into categories according to their effects (for example, healing, promoting vitality, or relieving pain). Modern pharmacy now deals with medicinal plants and getting excellent biological activity of those plants. Papaya leaf extract is derived from the leaves of papaya tree. Papaya tree is commonly found everywhere in Bangladesh. Papaya tree can said medicinal plant as it has proven several biological activity and active against several diseases. There are many health benefits of papaya leaf extract has found including micronutrient provider, red blood cell production, immune booster, antibacterial activity, antioxidant activity and aids in digestion (Rochway,2012). This paper is focusing on the antibacterial and antioxidant properties of papaya leaf extract.

Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns. These factors have inspired the widespread screening of plants for possible medicinal, antimicrobial and antioxidant properties. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease and in the aging process. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

1.2 Natural Products in History

Natural products (secondary metabolites) have been the most successful source of potential drug leads. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest. Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity. The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh) which are still used today to treat coughs, colds and inflammation. The Ebers Papyrus (2900 B.C.) is an Egyptian pharmaceutical record, which documents over 700 plant-based drugs ranging from gargles, pills, infusions, to ointments. The Chinese Materia Medica (1100 B.C.) (Wu Shi Er Bing Fang, contains 52 prescriptions), Shennong Herbal (~100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) are documented records of the uses of natural products. The Greek physician, Dioscorides, (100 A.D.), recorded the collection, storage and the uses of medicinal herbs, whilst the Greek philosopher and natural scientist, Theophrastus (~300 B.C.) dealt with medicinal herbs. During the Dark and Middle Ages the monasteries in England, Ireland, France and Germany preserved this Western knowledge whilst the Arabs preserved the Greco-Roman knowledge and expanded the uses of their own resources, together with Chinese and Indian herbs unfamiliar to the Greco-Roman world. It was the Arabs who were the first to privately own pharmacies (8th century) with Avicenna, a Persian pharmacist, physician, philosopher and poet, contributing much to the sciences of pharmacy and medicine through works such as the *Canon Medicinae* (NCBI).

According to Kapoor L.D. Ayurvedic medicine, also called Ayurveda, originated in India several thousand years ago. The term "Ayurveda" combines the Sanskrit words *ayur* (life) and *veda* (science or knowledge). Thus, Ayurveda means "the science of life."

There are two main re-organizers of Ayurveda whose works are still existing intact today Charak and Sushrut. The third major treatise is called the Ashtanga Hridaya, which is a

concise version of the works of Charak and Sushrut. Thus the three main Ayurvedic texts that are still used today are the Charak Samhita (compilation of the oldest book Atreya Samhita), Sushrut Samhita and the Ashtangha Hridaya Samhita. These books are believed to be over 1,200 years old. It is because these texts still contain the original and complete knowledge of this Ayurvedic world medicine, that Ayurveda is known today as the only complete medical system still in existence.

1.3 Drug Discovery in 20th century

The development of small molecule therapeutic agents for the treatment and prevention of diseases has played a critical role in the practice of medicine for many years. In fact, the use of natural extracts for medicinal purposes goes back thousands of years; however, it has only been in the past half century or so that searching for new drugs has found itself in the realm of science. In 1900, one-third of all deaths in the U.S. were from three general causes that are rare today because they are preventable and/or treatable: pneumonia, tuberculosis, and diarrhea. By 1940, the chance of dying from these three causes was 1 in 11; by 2000, the odds were down to 1 in 25 (NCBI, 2010).

Of the three, only pneumonia remains in the list of top ten causes of death, which is now led by more complex conditions such as cardiovascular disease and cancer. While other factors such as improved sanitation and vaccination certainly played a role in the increase of life expectancy during the twentieth century – from less than 50 years in 1900 to more than 77 years in 2000 – the availability of drugs to control infection, hypertension, hyperlipidemia, and to some extent even cancer, certainly also contributed to the obvious improvement in our collective health and life expectancy during that period (NCBI, 2010).

The history of drug discovery in the pharmaceutical industry and academic labs over the past half-century shows a progression of discovery paradigms that began shortly after “miracle drugs” such as the penicillins became available to the public after World War II. That same decade also saw the rise of synthetic organic chemistry, which had progressed to the point that the large scale preparation of “non-natural” drugs or drug candidates was economically feasible (NCBI, 2010).

1.4 Challenges to Natural Products-Based Drug Discovery

(NCBI, 2006)In spite of the success of the traditional approach to drug discovery by the bioactivity-directed fractionation of plant and marine extracts, this approach has not fared well in recent years, particularly in terms of funding from the major granting agencies in the U.S. and Europe, and in the support of this research within major pharmaceutical companies. The major reasons for this can be summarized as follows:

a. Incompatibility of Crude Natural Product Extracts with High-throughput Screening

Drug discovery within the pharmaceutical industry, with few exceptions, is based on the high throughput screening (HTS) of tens of thousands of compounds a week, using enzyme or receptor-based assays designed to uncover compounds with specific mechanisms of action.[50] This poses a dual problem for natural products screening. In the first place, crude natural product extracts are complex mixtures, containing hundreds of compounds, often including polyphenolic compounds such as plant tannins. Tannins act as promiscuous protein binders, and thus give false positive readouts in HTS, so that crude plant extracts cannot be used in HTS. Although this problem is solvable in principle by detanninization procedures,[51] a second problem then rears its head. Once a lead extract has been identified in natural products drug discovery, in the classical approach the active compound must be isolated by a process of bioactivity-directed fractionation, which can take weeks or months. HTS is not a good mechanism to use for this approach, because a typical HTS assay may be online for only a few weeks, and so the fractionation would need to be supported by another assay, adding cost to the process (NCBI, 2006).

a. Diversion of Resources to Combinatorial Chemistry

The increasing availability and sophistication of HTS from the early 1990's created the opportunity to screen libraries of hundreds of thousands or even millions of compounds, far larger than the existing compound libraries at most major pharmaceutical companies. This naturally created a demand for compounds to satiate the maw of the screening monster, and combinatorial chemistry provided the perfect fit, with its ability to generate libraries of tens of thousands of compounds. It was

seemingly a marriage made in heaven. Sadly, this approach has not been the panacea that it was hoped to be, and few drugs have been discovered by the combination of HTS and combinatorial chemistry. This lack of productivity is in part responsible for the decline in new drugs, with only 20 new drugs approved in the USA in 2007, down from an average of about 40 a year from 1981–2005.[38] Although the productivity of combinatorial chemistry as a drug discovery tool will no doubt eventually improve, as more importance is being placed on making "natural product like" compounds by diversity-oriented synthesis, the present situation has not changed significantly since 2004, when Ortholand and Ganesan could write: "The early years of combinatorial chemistry suffered from an excess of hype, and a major victim was natural-product screening. Many organizations went through an irreversible shift in policy, and prematurely discontinued their efforts in this area. We are now seeing the backlash from this knee-jerk reaction. The early combinatorial strategies were flawed and unproven, and have yet to deliver any blockbuster drugs. Meanwhile, we have lost the uniqueness of screening natural-product space as a complement to synthetic compounds. If past indicators are any guide, there are undoubtedly many more unique and potent biologically active natural products waiting to be discovered." A recent review by Ganesan concludes "one can only hope that natural products that have served as an important source of drugs in the past will not be overlooked in 21st century drug discovery"(NCBI, 2006).

b. Technical Difficulties

In addition to the problems with HTS noted above, the isolation of bioactive compounds from plants and marine organisms faces a number of technical challenges. These include the variability of the source material (since an activity found in one collection may be absent in another), the difficulty of isolating the active constituents, the possibility that the active compound is a known compound (thus not protectable by composition-of-matter patents), and the costs of collection. However, as will be discussed below, new methods and techniques offer exciting opportunities to avoid or at least ameliorate many of these difficulties(NCBI, 2006).

c. Resupply Problems

A further level of difficulty is encountered once a particular natural product has been isolated and identified as a lead compound, since this raises the large issue of compound supply. Depending on the potency of the compound and its target, several grams to hundreds of grams are needed for preclinical development, and multi-kilogram quantities would be needed for clinical use.

Probably the classic case of the problem of compound supply was with the anticancer drug paclitaxel, then known as taxol. The clinical activity of this compound against ovarian cancer was reported in 1989 and this touched off an intensive search for supplies for clinical use in what has been called the "taxol supply crisis". The problem was especially acute in the case of taxol because it treated a life-threatening disease but was obtainable at that time only from the bark of the western yew, *Taxus brevifolia*, which grew predominantly in the old-growth forests of the Pacific Northwest, home to the endangered spotted owl. The solution to this problem initially involved synthetic chemistry, as described below (NCBI, 2006).

A different kind of resupply problem arises when the plant itself is used as the medicinal agent, as is still the case for a large percentage of the world's population. In this case there is a real danger that non-sustainable harvesting will result in depletion of these critical resources, and initiatives are needed to commercialize the cultivation of the major species involved. This aspect of the supply problem is discussed in more detail by Cordell (NCBI, 2006).

d. Financial Pressures

On top of all the problems noted above, the pharmaceutical industry in general, and particularly in the USA, is undergoing a massive retrenchment, with major cuts in pharmaceutical research and development. As one analysis put it, "Big pharma's path through the recession is littered with job and program cuts and plant closures and lists numerous examples to back up this statement. These financial pressures make it very difficult for "Big Pharma" to invest the resources that would be needed to regain the effectiveness of their former natural product discovery programs. This in turn implies that developing nations cannot rely on "Big Pharma" to discover and develop their

medicinal natural product resources; this task must be undertaken by smaller and more nimble companies and by academic researchers (NCBI, 2006).

1.5 Medicinal Plant Of Bangladesh

Medicinal plants, plants used as natural medicines. This practice has existed since prehistoric times. There are three ways in which plants have been found useful in medicine. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents. Second, they may be used as agents in the synthesis of drugs. 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.Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethnobotanists, anthropologists, pharmaceutical chemists, and physicians. Many modern medicines had their origin in medicinal plants. Examples include aspirin from willow bark (*Salix spp.*), digitalis from foxglove (*Digitalis purpurea*), and vinblastine from Madagascar periwinkle (*Vinca rosea*) for the treatment of childhood leukemia.

A large group of plants used in medicine or veterinary practice for therapeutic or prophylactic purposes. The actual number of medicinal plants is not known, but there is no doubt that their number is very big at present. So a large number of plants with medicinal constituents have been described, the number of which presently stands at about one-sixth the number of the flowering plants so far pharmacologically evaluated.

In Bangladesh Unani and Ayurvedic medicines were being prepared from plants following the age-old traditional methods available in literature. These herbal medicines may thus be termed as upgraded herbal medicine or modern herbal medicines. The herbal medicine manufacturers of the modern herbal drugs are adopting scientific techniques to a great extent in order to keep the therapeutic value of the active constituents' intact following modern manufacturing process. In the manufacture of a particular medicine, several medicinal plants of similar efficacy are used with the consequent synergistic effect of the active constituents present in the plant materials.

Tab 1.1: Drugs of plant origin used in modern medicine

Common Name	Botanical Name	Use
Amla	*Emblica officinalis*	Vitamin C, Cough , Diabetes, Cold, Laxative, Hyper acidity.
Ashok	*Saraca Asoca*	Menstrual Pain, Terine, Disorder, Deiabetes.
Bael / Bilva	*Aegle marmelous*	Diarrrhoea, Dysentry, Constipation.
Chiraita	*Swertia Chiraita*	Skin diseases, Burning, Censationa, Fever
Kalmegh/ Bhui neem	*Andrographis Paniculata*	Fever, Weekness, Release of gas.
Long peeper / Pippali	*Peeper longum*	Appetizer, Enlarged spleen, Bronchities, Cold, Antidote.
Pashan Bheda / Pathar Chur	*Coleus barbatus*	Kidny stone, Calculus.
Sandal Wood	*Santalum Album*	Skin disorder, Burning, Sensation, Jaundice, Cough.
Satavari	*Asparagus Racemosus*	Enhance lactation, General weekness, Fatigue, Cough.
Senna	*Casia augustifolia*	General debility tonic, Aphrodisiac.
Tulsi	*Ocimum sanclum*	Cough, Cold, Bronchitis, Expectorand.
Pippermint	*Mentha pipertia*	Digestive, Pain killer.
Henna/ Mehendi	*Lawsennia iermis*	Burning, Steam, Anti-imflamatary.
Gritkumari	*Aloe Verra*	Laxative, Wound healing, Skin, Burns & Care, Ulcer.
Sada Bahar	*Vincea rosea*	Leaukamia, Hypotensive.
Vringraj	*Eclipta alba*	Anti-inflamatory, Digestive, Hairtonic.
Neem	*Azardirchata indica*	Sdedative, analgesic, Epilepsy.
Anantamool/sariva	*Hemibi smus Indicus*	Appetiser, Carminative, Aphrodisiac, Astringent.
Kantakari	*Solanum Xanthocarpum*	Diuretic, Antiinflamatory, Appetiser, Stomachic.
Shankhamul	*Geodorum denciflorum*	Antidiabetic.

At present, thousands of plant metabolites are being successfully used in the treatment of variety of diseases. A few striking examples of plant metabolites include taxol from *Taxus brevifalia*, vincristine and vinblastine from *Vinca rosea*, of which are important anticancer

agents being used clinically. In the current popular field of chemotherapy, cepharanthine, isolated is being used as a prophylactic in the management of tuberculosis.

In China, about 15,000 factories are involved in producing herbal drugs; Herbal medicines have been developed to a remarkable standard by applying modern scientific technology in many countries, such as China, India, Bangladesh, Sri Lanka, Thailand and United Kingdom. In these countries, the dependence of allopathic drugs has been described to greater extent.

"Modern medicine still has much to learn from the collector of herbs" said Dr. Hafdan Mohler, director general of World Health Organization. Many of the plants, familiar to the witch doctor really do have the healing power that tradition attaches to them. The age old art of the herbalist must be tapped.

1.6 The Plant Family Caricaceae

Caricaceae - a small family of flowering plants comprising about 35 species in six genera. *Carica papaya*, the family's most popular representative, is widely grown throughout the World's tropics. It is appreciated not only for its delicious and nutritive fruits, but also because it contains the enzyme papain, which is extensively used in medicines, as meat tenderizer, for softening textiles, silk, and leather, and in beer production. Several other species also have edible fruits and produce papain. For example, *Vasconcellea pubescens*, *Jacaratia spinosa*, and the hybrid *Vasconcellea* × *heilbornii* show promising characteristics for further economic exploitation and development of new crops (Herbaria, 1985).

Most members of Caricaceae are trees or shrubs (three *Jarilla* species from Mexico and Guatemala are herbs). All species produce latex that can be white or light yellow. Leaves vary from entire to deeply lobed or palmate. The flowers in Caricaceae are monoclinous (unisexual). Male flowers are mostly borne in an inflorescence with more than ten flowers; they have a tubular corolla, filled with sweet nectar; nectaries are located on a small pistillode (nonfunctional ovary); stamens are fused to the corolla throat and distributed in two pentamerous whorls. Female flowers are often solitary or bunched in few-flowered inflorescences (few species present congested female inflorescences); they are devoid of nectar; petals are not fused (with few exceptions); ovaries are divided into one or five chambers (locules); there are five stigmas that are either entire or bifurcated. Fruits are berries with many seeds. The seeds are surrounded by a mucilaginous aril; the testa can be

ornamented or not. Caricaceae and its sister family Moringaceae are part of the mustard-oil plant order (Brassicales), which also comprises 15 other families of flowering plants, including the Brassicaceae, the cabbage family. Moringaceae is a small family with 13 species distributed in southeastern Asia and Africa (Herbaria, 1985).

Tab 1.2: **Different Plants of Caricaceae Family**

Species	**Common Name**	**Picture**
Vasconcellea pubescens	Mountain papaya	

Vasconcellea sprucei	Not found	
Vasconcellea goudotina	Papayuelo	
Carica quercifolia	Oak-Leaved papaya	

Carica dodecaphylla	Tree papaya, Deer's papaya, Thorny papaya	
Jacaratia corumbensis	Jacaratia	
Jacaratia digitata	Jacaratia	

Jacaratia spinosa	Wild papaya	

(davesgarden.com 2010)

1.7 Intoduction to *Carica Papaya*

1.7.1 Scientific classification of Carica papaya

Kingdom: Plantea

Subkingdom: Viridaeplantae

Infraingdom: Steotophyta

Division: Magnoliophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: Rosanae

Order: Brassicales

Family: Caricaceae

Genus: *Carica*

Species: *Carica papaya* (IT IS)

Binomial Name: *Carica Papaya*

1.7.2 Plant Description

The papaya, *Carica papaya* L., is a member of the small family Caricaceae allied to the Passifloraceae. As a dual- or multi-purpose, early-bearing, space-conserving, herbaceous crop, it is widely acclaimed, despite its susceptibility to natural enemies (hort.).

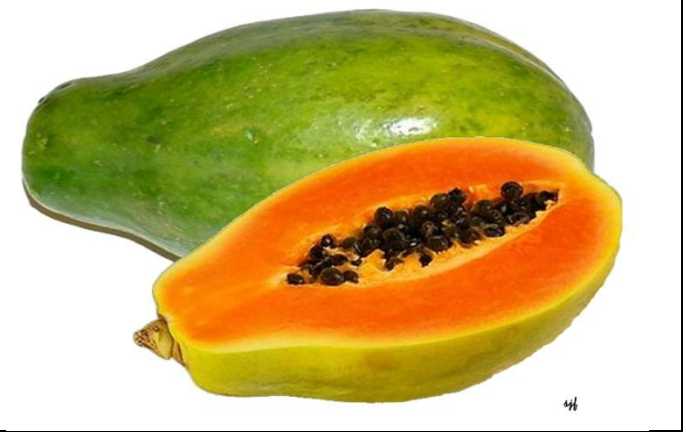
In some parts of the world, especially Australia and some islands of the West Indies, it is known as papaw, or pawpaw, names which are better limited to the very different, mainly wild *Asimina triloba* Dunal, belonging to the Annonaceae. While the name papaya is widely recognized, it has been corrupted to *kapaya, kepaya, lapaya* or *tapaya* in southern Asia and the East Indies. In French, it is *papaye* (the fruit) and *papayer* (the plant), or sometimes

figuier des Iles. Spanish-speaking people employ the names *melón zapote, lechosa, payaya* (fruit), *papayo* or *papayero* (the plant), *fruta bomba*, *mamón* or *mamona*, depending on the country. In Brazil, the usual name is *mamao*. When first encountered by Europeans it was quite naturally nicknamed "tree melon" (hort).

Papaya is a short-lived perennial growing to 30ft (9.14m) high. It hollow, herbaceous stem is usually unbranched. The deeply lobed, palmate leaves are borne on long, hollow petioles emerging from the stem apex. Flowers occur in leaf axils. Older leaves die and fall as the tree grows. Papaya flowers are fragrant and have five cream white to yellow orange petals 1 to 2 in (2.3 to 5.1 cm) long. The stigmatic surface are pale green and the stamens are bright yellow.(Hawaii) Papaya fruits are smooth skinned. They vary widely in size and shape, depending on verity and type of plant. Female plants of Solo verities usually produce round fruits. Other papaya verities produce various shaped fruits which may weigh up to 20lb (9.1 kg). the fruit usually contain many seeds surrounded by a smooth yellow to orange red flash that is sweet in good verities.

Tab 1.3: Different parts of *Carica papaya*

Plant Part	Picture
Papaya plant	

Papaya leaf	
Papaya fruit	

1.7.3 Uses of *Carica papaya*

Ripe papayas are most commonly eaten fresh, merely peeled, seeded, cut in wedges and served with a half or quarter of lime or lemon. Sometimes a few seeds are left attached for those who enjoy their peppery flavor but not many should be eaten. The flesh is often cubed or shaped into balls and served in fruit salad or fruit cup. Firm-ripe papaya may be seasoned and baked for consumption as a vegetable. Ripe flesh is commonly made into sauce for shortcake or ice cream sundaes, or is added to ice cream just before freezing; or is cooked in pie, pickled, or preserved as marmalade or jam. Papaya and pineapple cubes, covered with sugar sirup, may be quick-frozen for later serving as dessert. Half-ripe fruits are sliced and crystallized as a sweetmeat.

Papaya juice and nectar may be prepared from peeled or unpeeled fruit and are sold fresh in bottles or canned. In Hawaii, papayas are reduced to puree with sucrose added to retard gelling and the puree is frozen for later use locally or in mainland USA in fruit juice blending or for making jam.

Unripe papaya is never eaten raw because of its latex content. [Raw green papaya is frequently used in Thai and Vietnamese cooking.] Even for use in salads, it must first be peeled, seeded, and boiled until tender, then chilled. Green papaya is frequently boiled and served as a vegetable. Cubed green papaya is cooked in mixed vegetable soup. Green papaya is commonly canned in sugar sirup in Puerto Rico for local consumption and for export. Green papayas for canning in Queensland must be checked for nitrate levels. High nitrate content causes detinning of ordinary cans, and all papayas with over 30 ppm nitrate must be packed in cans lacquered on the inside. Australian growers are hopeful that the papaya can be bred for low nitrate uptake.

A lye process for batch peeling of green papayas has proven feasible in Puerto Rico. The fruits may be immersed in boiling 10% lye solution for 6 minutes, in a 15% solution for 4 minutes, or a 20% solution for 3 minutes. They are then rapidly cooled by a cold water bath and then sprayed with water to remove all softened tissue. Best proportions are 1 lb (.45 kg) of fruit for every gallon (3.8 liters) of solution.

Young leaves are cooked and eaten like spinach in the East Indies. Mature leaves are bitter and must be boiled with a change of water to eliminate much of the bitterness. Papaya leaves contain the bitter alkaloids, carpaine and pseudocarpaine, which act on the heart and respiration like digitalis, but are destroyed by heat. In addition, two previously undiscovered major D^1-piperideine alkaloids, dehydrocarpaine I and II, more potent than carpaine, were reported from the University of Hawaii in 1979. Sprays of male flowers are sold in Asian and Indonesian markets and in New Guinea for boiling with several changes of water to remove bitterness and then eating as a vegetable. In Indonesia, the flowers are sometimes candied. Young stems are cooked and served in Africa. Older stems, after peeling, are grated, the bitter juice squeezed out, and the mash mixed with sugar and salt.

In India, **papaya seeds** are sometimes found as an adulterant of whole black pepper. Collaborating chemists in Italy and Somalia identified 18 amino acids in papaya seeds, principally, in descending order of abundance, glutamic acid, arginine, proline, and aspartic acid in the endosperm; and proline, tyrosine, lysine, aspartic acid, and glutamic acid in the sarcotesta. A yellow to brown, faintly scented oil was extracted from the sundried, powdered seeds of unripe papayas at the Central Food Technological Research Institute, Mysore, India. White seeds yielded 16.1% and black seeds 26.8% and it was suggested that the oil might have edible and industrial uses (hort).

Food Value

The papaya is regarded as a fair source of iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The following figures represent the minimum and maximum levels of constituents as reported from Central America and Cuba.

Tab 1.4: Food Value Per 100 g of Edible Portion

	Fruit	*Leaves*
Calories	23.1-25.8	
Moisture	85.9-92.6 g	83.3%
Protein	.081-.34 g	5.6%
Fat	.05-.96 g	0.4%
Carbohydrates	6.17-6.75 g	8.3%
Crude Fiber	0.5-1.3 g	1.0%
Ash	.31-.66 g	1.4%
Calcium	12.9-40.8 mg	0.406% (CO)
Phosphorus	5.3-22.0 mg	
Iron	0.25-0.78 mg	0.00636%
Carotene	.0045-.676 mg	28,900 I.U.
Thiamine	.021-.036 mg	
Riboflavin	.024-058 mg	
Niacin	.227-555 mg	
Ascorbic Acid	35.5-71.3 mg	38.6%
Tryptophan	4-5 mg	
Methionine	1 mg	
Lysine	15-16 mg	
Magnesium		0.035%
Phosphoric Acid		0.225%

Carotenoid content of papaya (13.8 mg/100 g dry pulp) is low compared to mango, carrot and tomato. The major carotenoid is cryptoxanthin.

Papain

The latex of the papaya plant and its green fruits contains two proteolytic enzymes, papain and chymopapain. The latter is most abundant but papain is twice as potent. In 1933, Ceylon

(Sri Lanka) was the leading commercial source of papain but it has been surpassed by East Africa where large-scale production began in 1937.

The latex is obtained by making incisions on the surface of the green fruits early in the morning and repeating every 4 or 5 days until the latex ceases to flow. The tool is of bone, glass, sharp-edged bamboo or stainless steel (knife or raxor blade). Ordinary steel stains the latex. Tappers hold a coconut shell, clay cup, or glass, porcelain or enamel pan beneath the fruit to catch the latex, or a container like an "inverted umbrella" is clamped around the stem. The latex coagulates quickly and, for best results, is spread on fabric and oven-dried at a low temperature, then ground to powder and packed in tins. Sun-drying tends to discolor the product. One must tap 1,500 average-size fruits to gain 1 1/2 lbs (0.68 kg) of papain.

The lanced fruits may be allowed to ripen and can be eaten locally, or they can be employed for making dried papaya "leather" or powdered papaya, or may be utilized as a source of pectin.

Because of its papain content, a piece of green papaya can be rubbed on a portion of tough meat to tenderize it. Sometimes a chunk of green papaya is cooked with meat for the same purpose (hort).

Folk Uses

In tropical folk medicine, the fresh latex is smeared on boils, warts and freckles and given as a vermifuge. In India, it is applied on the uterus as an irritant to cause abortion. The unripe fruit is sometimes hazardously ingested to achieve abortion. Seeds, too, may bring on abortion. They are often taken as an emmenagogue and given as a vermifuge. The root is ground to a paste with salt, diluted with water and given as an enema to induce abortion. A root decoction is claimed to expel roundworms. Roots are also used to make salt.

Crushed leaves wrapped around tough meat will tenderize it overnight. The leaf also functions as a vermifuge and as a primitive soap substitute in laundering. Dried leaves have been smoked to relieve asthma or as a tobacco substitute. Packages of dried, pulverized leaves are sold by "health food" stores for making tea, despite the fact that the leaf decoction is administered as a purgative for horses in Ghana and in the Ivory Coast it is a treatment for

genito-urinary ailments. The dried leaf infusion is taken for stomach troubles in Ghana and they say it is purgative and may cause abortion (hort).

Chapter -2
Literature Review

2.1 Characteristics and Importance of *Carica papaya*

Papaya (*Carica papaya* L.) is a member of Caricaceae Family. This family comprises of 31 species in the four genera: three genera from America (Carica, Jacaritia and Jarilla) and one from equatorial Africa (Cylicomorpha). Papaya is an economically important fruit crop in Hawaii, Australia, India, Srilanka, Phiilipines and South-east Asia including Thailand. It is also known as papaw, pawpaw, papayer (French), melonenbaum (German), lechosa (Spanish), mamao, mamaociro (Portuguese), mugua (Chinese) and malakol (Thailand) (Nakasone & Paull 1998).

The origin of *Carica papaya* is in Tropical America. Its seeds were distributed from the Caribbean to Malacca and India by travellers and botanists in the eighteenth century. The distribution was continued throughout Asia and Pacific. Carica papaya is grown in all tropical countries and many subtropical countries between 32 °North and South latitudes but the high commercial production is found between 23 °N and S latitudes (Nakasone & Paull 1998).

Characteristics of Stem:

Carica papaya is a fast-growing tree herbaceous like plant 5-7 meters in height. Papaya normally has a monaxial stem without branching but it has multi-stems when damaged. When the stem is wounded white milky latex oozed from the wound. Although papaya can be up to 9 meters height, it is easily damaged and makes harvesting of fruit difficult (Nakasone & Paull 1998).

Characteristics of Leaves:

The cluster of leaves at the apex and along the upper of the stem makes up the foliage on tree. New leaves emerge from the apex and old leaves senescence and fall. Leaves are palmately lobed with prominent venation; the blade is deeply divided into 7-11 segments and can measured 40-50 cm in diameter with 15 mature leaves per plant. The leaves contain white milk latex (Nakasone & Paull 1998).

Characteristics of Flower:

Papaya flowers are born in florescences which appear in the axils of leaves. It can be female, male or hermaphrodite flowers. Female flowers are held close against the stem as single flowers or in cluster of 2-3 flowers. Male flowers are smaller and more numerous. Hermaphrodite (perfect) flowers are intermediate between the female and male (Nakasone & Paull 1998).

Characteristics of Fruit:

The fruit superficially resembles a melon puriform, oval and elongated in shape. The fruits range in size from 7-30 cm. The fruit is normally composed of 5 carpels. Fruits from female trees are spherical whereas the shape of fruits from hermaphrodite trees is affected by environmental factors that modify floral morphology during early development of the inflorescence. Green fruits contain an abundance of milky latex. Ripe fruits have yellow-orange coloured skin. Mature fruits contain numerous grey-black spherical seeds 5 mm in diameter (Nakasone & Paull, 1998).

Importance:

Papaya is mainly cultivated for its edible fruits as a fresh fruit and for use of drinks, jams, candies and dried fruit. Ripe fruits are usually eaten fresh and green fruits are also used as a cooked vegetable. Papaya also has several industrial uses. Biochemically, its leaves and fruits produce several proteins and alkaloids with important medical and industrial application. The latex of green fruits contain a proteolytic enzyme, papain, used in the beverage, food and pharmaceutical industries for production of chewing gum, chill-proofing beer, tenderising meat, treat digestive disorders, degum natural silk, extracted fish oil. It is also used in the cosmetic industry, in soap, shampoo and face lifting preparations (Nunez, 1982). Evolutionary, papain may be associated with protection from frugivorous predators and herbivores (Australian Government, Department of Health and Ageing, 2003).

Papaya is a wholesome fruit. Papaya has more carotene compare to other fruits such as apples, guavas, sitaphal and plantains (Mumtaz, 2005). The fruit in 100 grams contains protein (1.0 g), carbohydrate (13.5 g) and fibre (0.5 g). It is a good source of minor such as Calcium (31.0 mg), Potassium (337.0 mg) and Magnesium (0.8 mg).

2.2 Antibacterial activity of *Carica papaya* leaf

The antimicrobial activity of the plant extract against microorganism examined was assessed by the presence or absence of inhibition zones. The result showed that the ethanol extract of the plant leaves (Table 1) demonstrated better antimicrobial activity compared to water extract which produced inhibition against three of the tested bacteria (*B. cereus, S. dysenteriae and Salmonella typhi*) within the range of 4.0mm-10.0mm. On the other hand, the hexane extract of the plants produced no antimicrobial activities. Table 1 revealed that the

ethanol extract of *C. papaya* leaf and *A. occidentale* leaf showed antimicrobial activity against the eight microorganisms tested; *C. nucifera* leaf inhibited six out of eight microorganisms while *C. sinesis* and *C. limon* leaf exhibited antimicrobial activity against five microorganisms. The highest inhibition zone of 12mm was observed in *A. occidentale* leaf against *Shigella dysenteriae* while *C. limon* leaf had the lowest inhibition zone of 2mm against *B. cereus*. Plants having significant medicinal values have often been found to be rich in phenolics and to have high antioxidant potentials. Based on the results it can be concluded that the antimicrobial nature of ethanol extract of *A. occidentale* leaf is apparently related to its high phenolic content. The antioxidant and antimicrobial activities recorded from cashew leaf may be as a result of the earlier report which documented a wide range of chemicals such as anacardic acids, anacardol, hydroxybenzoic acid, kaempferol, salicylic acid and tannins isolated and identified from this plant. Furthermore, the isolation of antibacterial phenolic compounds such as anacardic acids, cardols, methylcardols and cardanols from cashew nut shell oil. The antifungal, antiaflatoxigenic and antioxient activity of *C. sinensis* is equally reported. Flavonoid, phenolic compound, tannins and alkaloid are the most important antimicrobial agent and bioactive constituents in plant. Some of these bioactive compounds singly or in combination inhibit the life processes of microorganisms by binding their protein molecules, acting as chelating agents, altering their biochemical systems, or causing inflammation of the cells. Furthermore, the bitter taste, pungent and repulsive smell in some plants have been found to have repressive ability over the metabolic activities of microorganisms (Elisa et al, 2011).

Table 2.1: Antimicrobial activity of ethanol extract (0.1 mg/ml) from the leaf samples (Elisa. et al, 2011).

Method: modified agar-well diffusion					Zone of inhibition (mm)	
Microorganism	***Anacardium occidentale***	***Cocos nucifera***	***Citrus Sinesis***	***Citrus limon***	*Carica papaya*	Control (Ethanol)
Acinetobacter spp.	10.0	8.0	-	3.6	6.0	-
Bacillus cereus	10.0	7.0	2.0	4.0	5.0	-
Escherichia coli	10.0	3.0	4.0	2.0	4.0	-
Shigella dysenteriae	12.0	5.0	5.0	5.0	6.0	-
Staphylococcus Aureus	10.0	-	-	-	5.0	-
Salmonella	7.0	3.0	4.0	2.0	4.0	-

typhi						
Aspergillus niger	6.0	3.0	9.0	-	10.0	-
Aspergillus flavus	2.0	-	-	-	10.0	-

(-)No inhibition

The results from the study show that paw-paw extracts have antibacterial activity. This is probably the reason why some people use paw-paw for the treatment of wounds in the villages.

The extract is bacteriostatic on and bactericidal on but has no effect on. This somewhat contradicts the findings of Vieria, where it was shown that the extract was bactericidal. The variation could have resulted to the concentration of ethanol used. In this study, 30% ethanol was used for extraction while 50% ethanol was used by Vieri. The higher concentration of ethanol may have produced a more potent extract that was bactericidal to both gram positive and gram negative organisms. It is worthy of note that Ethanol extract was more potent than distilled water extract.

This may be due to the fact that ethanol is a better solvent for extraction than water. The extract from seed, epicarp and endocarp of both ripe and unripe fruits had antibacterial activity but that from endocarp of unripe fruit had the most activity. This could be due to its high content of papain latex. It was also observed that the antibacterial activity of the extracts were relatively unaffected by temperatures between 30 C 50 C and this is commendable as it falls within the body temperature. It is believed that with rising degree of antimicrobial resistance against the commonly available and affordable antimicrobials, research should shift to the affordable alternatives which include the use of roots, herbs, fruits, etc for the treatment of common ailments(ajol).

(Elisa et al, 2011) It was reported that the extracts of papaya leaves could inhibit the growth of Rhizopus stolonifer. Antibacterial activity of Carica papaya leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol, ethyl acetate, and hexane. Papaya leaf extracts were tested against Bacillus stearothermophilus, Listeria monocytogenes, Pseudomonas sp., and Escherichia coli by agar diffusion method. The objectives of this study were to determine extract ability against pathogenic bacteria, to observe the influence of pH, NaCl, and heat on extracts ability, and to observe extract ability against B. stearothermophilus spores. The data showed that ethyl acetate extract could inhibit B. stearothermophilus, L. monocytogenes,

Pseudomonas sp., and E. coli. The extract activity was influenced by pH, and it was more effective in low pH. The extract activity was influenced by NaCl against B. stearothermophillus and E. coli. However, it was not influenced by NaCl in bioassay against L. monocytogenes and Pseudomonas sp. The extract activity was influenced by heating process against all the bacteria tested. The extracts inhibited B. stearothermophilus spores as well. Papaya leaves are potential natural anti-bacteria, which might be used in certain kinds of food.

formulation comprising of *C. papaya* roots, *M. indica* leaves, *Citrus limon* fruit and *C. citratus* leaves has also been reported to possess antibacterial activity against *S. typhi, S. paratyphi* and *S. typhimurium* (Nkuo-Akenji *et al*., 2001). Although various types of compounds have been identified from the leaves, bark, fruits, barks, roots, seeds and latex of *C. papaya* (Bennett *et al*., 1997; MacLeod and Pieris, 1983; Pousset *et al*., 1981; Sandhya and Veerannah, 1996; Schwab and Schreier, 1988; Sheu and Shyu, 1996; Tang 1979; Winterhalter *et al*., 1986), there has been no report on the chemical constituents of its flowers. *C. papaya* contains various types of biologically active compounds with the two important compounds are chymopapain and papain (Brocklehurst and Salih, 1985), which are thought to aid in digestion. Furthermore, papain also has been used in the treatment of arthritis. Among the various types of bioactive compounds isolated from *C. papaya*, alkaloids, carpaine, dehydrocarpaines, flavonols, tannins and benzyglucosinolate have been reported to be presence in the leaves while linalool, cis- and trans-linalool oxide, α-linolenic acid, α-phellandrene, α- and γ-terpinenes, 4-terpineol, terpinolene have been reported to be presence in the fruits as mentioned earlier. It is plausible to suggest the involvement of mangiferin, at least in part, in the antibacterial activity of *M. indica* based on the previous reports mentioned earlier. However, it was not possible to link any of the compounds isolated from various parts of *C. papaya* as described earlier with the observed antibacterial activity since there was no report on the chemical constituents of its leaves. However, their present and possibility of occurrence in the flowers of *C. papaya* should not be excluded. The present study has proven that the respective *M. indica* and *C. papaya* leaves and flowers possessed antibacterial activity and thus provide the initial steps for future isolation and identification of antibacterial agents from those plant.

Tab 2.2: The antibacterial activity of methanol and ethanol extracts of *Carica papaya* flowers determined by disc diffusion method

	Concentration (%)							
	MECP				EECP			
Bacteria	12.5	25	50	100	12.5	25	50	100
C. diptheriae	-	+	+	++	-	-	-	+
S. aureus	++	++	+++	+++	+	++	+++	+++
S. pneumoniae	+++	+++	+++	++++	++	++	+++	+++
S. typhi	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-

2.3 Antioxident activity of *Carica papaya* leaf

The reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical and reduction of this chemical by probable antioxidants result in loss of absorbance. Thus, the degree of discolouration of the solution indicates the scavenging efficiency of the added substance. The results of free radical scavenging properties of the extracts expressed in percentage DPPH activities are shown in Fig. 2. All the plant extracts exhibited moderate to high antioxidant activities. From ethanolic extract highest antioxidant activity was observed in *C.sinesis* leaf (56.79%) followed by *C. nucifera* leaf (45.28%), *C. papaya* leaf (42.59%), *A. occidentale* leaf (39.55%) and *C. limon* (32.75%). For hexane extract the highest antioxidant activity was obtained from *C. papaya* leaf (76.05%) while the least scavenging property (45.03%) was from *C. sinesis* leaf. Ability of water extracts from the tested leaves showed that the radical scavenging property ranged from 45.82% from *C. papaya* to 71.7% in *A. occidentale* leaf. However, the values obtained for phenol content and radical scavenging activity of leaves investigated in this study were lower when compared to that reported for some green leafy vegetables commonly consumed in Nigeria. Sun drying of the green leafy vegetables led to a significant increase in the total phenol content (6.45-223.08% gain), and free radical scavenging ability (126.00-5757.00% gain) (Elisa et al, 2011).

Natural phenolic exert beneficial effects mainly through their antioxidant activity. These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radical, such as hydroxyl radicals, chelating metal ion catalyst, decomposing primary product of oxidation to non radical specie and breaking chains to prevent continued hydrogen abstraction from substance [18]. In addition, polyphenolic compounds are primarily responsible for the antioxidant activity of natural extract due their redox properties and chemical structures. Several researches have demonstrated that there can be a correlation between phenolic content and antioxidant capacity of plant extracts or their essential oils. However, other bioactive compounds such as naphtoquinones, carnosic acid and carnosol, capsaicinoids compounds and allicin from garlic are considered as free-radical scavengers.

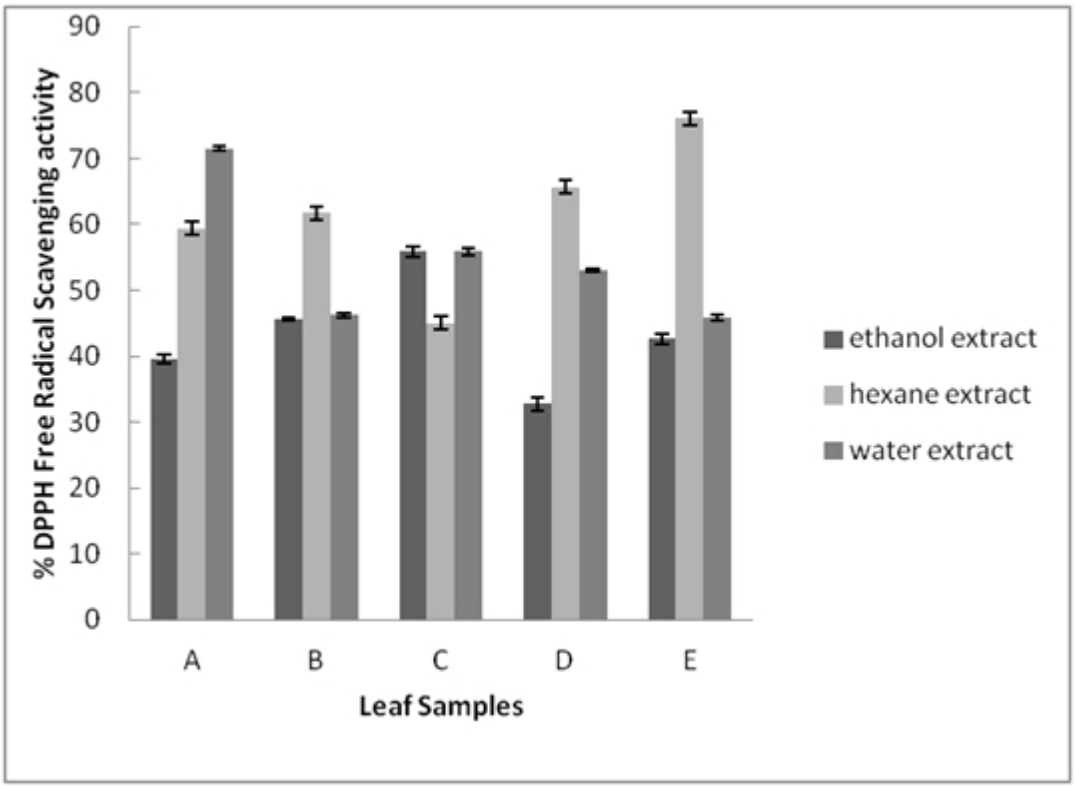


Figure 2.1: **Free Radical Scavenging activity of ethanol, hexane and water extracts from leaf samples A (*Anacardium occidentale*), B (*Cocos nucifera*), C (*Citrus sinesis*), D (*Citrus limon*) and E *(Carica papaya*) (Elisa et al, 2011)**

The leaves extract of *Magnifera indica* and *Carica papaya* revealed the presence of flavonoids, polyphenols and tannins. The antioxidant effect of plant products is mainly due to

radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins (Rahman and Moon, 2007).

The antioxidant activity of both *Mangifera indica* and *Carica papaya* showed high antioxidant activity at the same time at 50 min of extraction time with 89.70±0.03 and 86.95±0.07. The antioxidant activity of *C. papaya* was at highest level at 50 min with 89.70±0.03 and that of *M. indica* was at 30 and 50 min with 86.95±0.03 and 86.95±0.07, respectively as shown in Table 1 and Fig. 2 (Maisarahand, 2013)

Phenolic antioxidants are potent free radical terminators. The high potential of phenolics to scavenge free radicals may be due to the many phenolic hydroxyl groups. The aqueous extract of plants leaves demonstrated maximum antioxidants activity. Many plants extract exhibit efficient antioxidant properties due to their phtocontituents including phenolics. The antioxidant activity of the polphenol extract of *M. indica* has highest level at the dose of 100 μg mL^{-1} with 13.96±14.92 while that of *C. papaya* was at dose of 150 μg mL^{-1} as shown in Table 2.

Polyphenol is capable of acting as an antioxidant through many mechanisms available *in vitro* primarily as potent scavenger of free radical. The antioxidant activity of both *M. indica* and *C. papaya* was low at the same dose of 100 (μg mL^{-1}) with 8.65±13.73 and 23.18±8.11 as shown in figure 2.2.

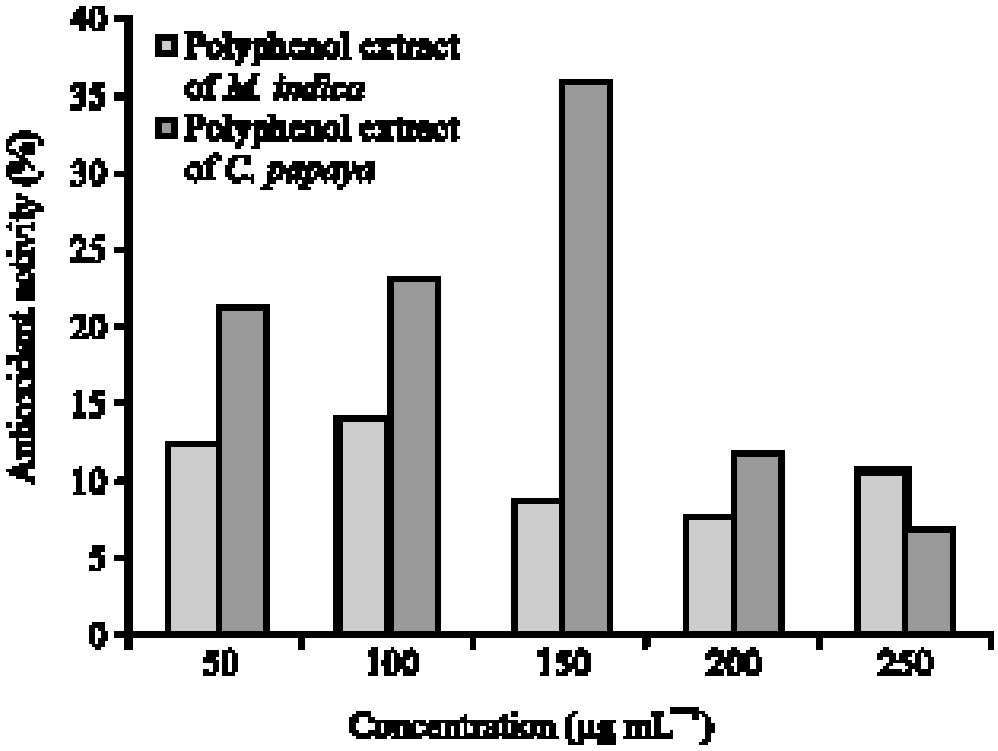


Figure 2.2: The bar chart of antioxidat of polyphenol extract of *M. indica* and *C. papaya(*medwelljournals)

The relative *in vitro* antioxidant and free radical scavenging activities of the extract and ascorbic acid were shown in Figure 1. It was observed that the extract possessed significant hydroxyl radical scavenging, hydrogen peroxide scavenging, metal chelating, and reducing abilities when compared with ascorbic acid. However, the abilities increased with concentration (P<0.05). The antioxidant ability of ascorbic acid (0.5 mg/mL) was higher than the equivalent concentration of the extract. The same trend was observed for the antioxidant potential using the phospomolybdate method. The ability of the extract to inhibit hydrogen peroxide induced erythrocyte haemolysis and lipid peroxidation was also concentration dependent (P<0.05) . However, ascorbic acid was better at inhibiting the hydrogen peroxide induced alterations as shown by the IC_{50} values.

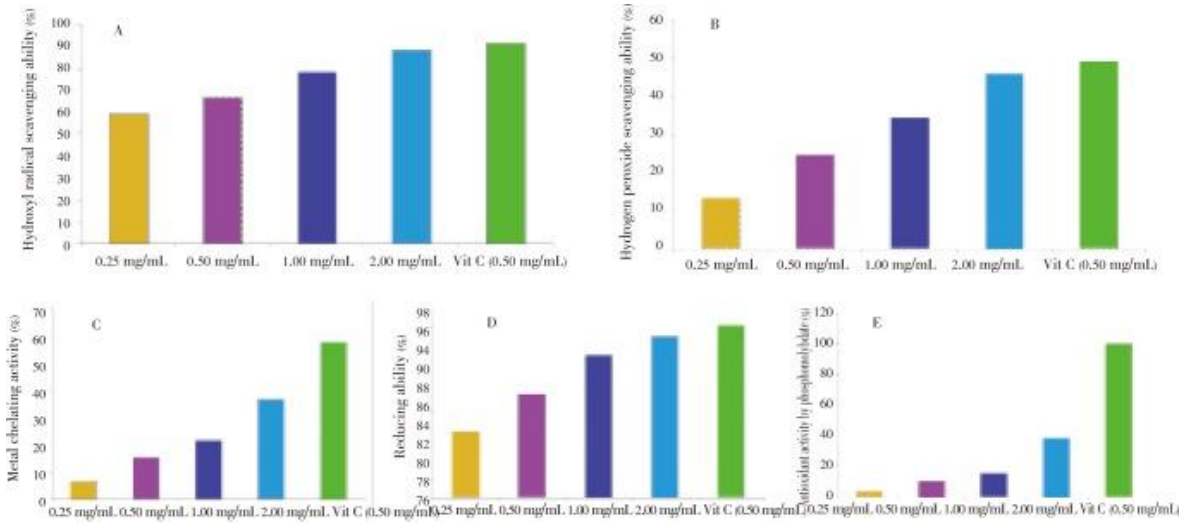


Figure 2.3: Relative antioxidant and free radical scavenging activities of *C. papaya* leaf extract and ascorbic acid (Vit C, 0.50 mg/mL) using various *in vitro* models(ncbi.nlm).

Chapter-3
Method

3.1 Method of study

This study was designed to isolate methanolic extract as well as to observe biological activities of the extract of *Carica papaya* (Family: Caricaceae). The study protocol consisted of the following steps:

- Cold extraction of the powdered stem bark of the plant with methanol at room temperature.
- Filtration and solvent evaporation of the methanolic crude extract.
- Performing antimicrobial test.
- Performing antioxidant test by DPPH free radical scavenging assay.

3.2 Preparation of plant sample

The leaves of *Carica papaya* were sun dried for several days. After complete drying the dried leaves were then ground in coarse powder using high capacity grinding machine in the Pharmacological Research Laboratory, Faculty of Pharmacy, East West University. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, and dry place for future use.

3.3 Extraction of the plant material

Total 300.18 gm *Carica papaya* leaf powder was finally obtained and about 200 gm of powdered leave material was taken in clean, round bottomed flask and macerated at room temperature in 2 liters of methanol for 7 days with occasional shaking for better extraction. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper. After filtration the filtrate was concentrated at 40°C with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue.

3.4 Antimicrobial Activity test

Worldwide, infectious disease is one of main causes of death accounting for approximately onehalf of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. It is estimated that

infectious disease is the underlying cause of death in 8% of the deaths occurring in the US (Pinner *et al*., 1996).

This is alarming given that it was once believed that we would eliminate infectious disease by the end of the millennium. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most dramatic increases are occurring in the 25–44 year old age group (Pinner *et al*., 1996).

These negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. It is this last solution that would encompass the development of new antimicrobials (Fauci, 1998).

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by any of the following three methods:

- Disc diffusion method
- Serial dilution method
- Bioautographic method

3.5 Principle of disc diffusion method

Solutions of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette.

Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control.

These plates are then kept at low temperature (4 0C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel (Barry, 1976).

As a result there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then incubated at 37 0C for 24 hours to allow maximum growth of the organisms. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms and a clear, distinct zone of inhibition will be visualized surrounding the medium.

The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and the mean of the readings is required (Bayer et al., 1966).

Figure 3.1: Disc diffusion method

3.6 Apparatus, reagent and test sample

- Filter paper disc
- Nutrient Agar Medium
- Petri dishes
- Candle
- Sterile forceps
- Inoculating loop

- Spreader
- Autoclave machine
- Laminar air flow hood
- Incubator
- Refrigerator
- Pipette
- Micropipettes
- Test tubes
- Test tube holders

3.7 Test materials of *Carica papaya*

- Methanolic crude extract of *Carica papya* leaves.
- 3 different concentration of *Carica papaya* leaves were placed in the disc.
- Each disc contains 50 μg/disc, 100 μg/disc and 150 μg/disc extract respectively.

3.8 Test microorganisms

The bacterial stains used for this experiment were collected from East West University. Test organisms are given below:

Tab 3.1: List of Bacteria

Name of Bacteria	**Type of bacteria**
Escherichio coli	Gram negative
Shigella dysenteria	Gram negative
Salmonella parathyphi	Gram negative
Staphylococcus aureus	Gram positive

3.9 Culture medium and their composition

The following media is used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms.

Tab 3.2: Composition of nutrient agar medium

Ingradient	Amount
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water	Qs 100ml
pH	7.2 ± 0.1 (at 250C)

3.10 Preparation of medium

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 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 1210C for 20 minutes.

3.11 Sterilization procedures

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 glassware's were sterilized by autoclaving at a temperature of 121 0C and a pressure of 15-lbs. /sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc.were also sterilized.

3.12 Preparation of subculture

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

3.13 Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10ml 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 and fungal suspension was immediately transferred to the sterilized petridishes.

3.14 Standard discs

These 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) disc was used as the reference.

Tab 3.3: Preparation of sample discs with test samples

Concentration of sample (g/ml)	**Dose (µg/disc)**
0.025	50
0.05	100
0.075	150

3.15 Application of the test samples

Standard Kanamycin (30 mg/disc) 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 produced by the test sample.

3.16 Diffusion 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 and fungi. The plates were then kept in an incubator for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium.

3.18 DPPH Free radical Scavenging activity

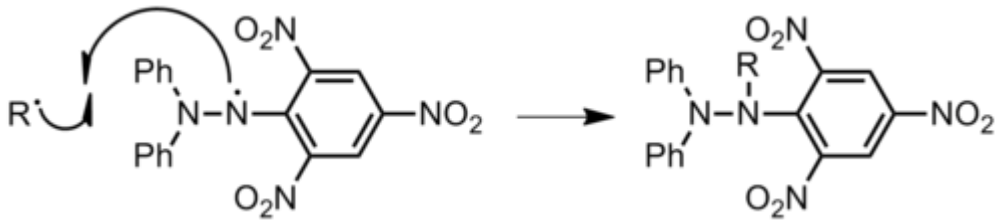
There is a considerable recent evidence that free radical induce oxidative damage to biomolecules. This damage causes atherosclerosis, aging, cancer and several other pathological events in living organisms (Halliwell *et al.,* 1992). Antioxidants which scavenge free radicals are known to possess an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and disease (Steinmetz and potter, 1996; Arouma, 1998; Bandonience *et al.,* 2000; Pieroni *et al.,* 2002; Couladis *et al.,* 2003). A number of reports on the isolation and testing of plants derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids (Shahidi *et al.,* 1992; Velioglu *et al.*, 1998; Pieta *et al.,* 1998). Dietary food contains a wide variety of free radical-scavenging antioxidants; for example, flavonoids and antioxidant vitamins such as ascorbic acid and ∞-tocopherol. These compounds are particularly rich in vegetables, fruits, tea, and wine. Epidemiological studies have shown that higher intake of fresh vegetables, fruits, tea and wine is associated with lower risk of mortality from cancer and coronary heart diseases. There is currently strong interest in natural antioxidants and their role in human health and nutrition.

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as butylated hdroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) are widely used as food additives to increase self life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, BHT and BHA are known to have not only toxic and carcinogenic effects and humans (Ito *et al*., 1986; Wichi, 1988), but abnormal effects on enzyme systems (Inatani, Nakatani & Fuwa *et al.,* 1983). Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakasha & Jaganmohan Rao., 2000).

3.19 Principle Of DPPH Free Radical Scavenging Activity

The present study was aimed at evaluating the *in vitro* free radical scavenging activity of *Carica papaya* (leaves) using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the method of Brand- Williams *et al.,* 1995. 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorption of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH (DPPH-H). DPPH radical scavenging activity is described as IC50 which is the concentration of samples to produce 50% reduction of the DPPH.



DPPH DPPH-H

Figure 3.2: Mechanism of free radical scavenging activity

3.20 Method

DPPH was used to evaluate the free radical scavenging activity of various compounds and medicinal plants (Choi *et al.*, 2000; Desmarchelier *et al.*, 1997).

- 50µl of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH.
- After 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm.
- Inhibition free radical DPPH in percent *%* scavenging activity was calculated as follows:

$$\% \text{ scavenging activity} = \frac{Ao - A1}{Ao} \times 100$$

- Where A blank is the absorbance of the control reaction (containing all reagents except the test material).
- Extract concentration providing 50% inhibition *(IC50)* was calculated from the graph plotted inhibition percentage against extract concentration.
- Ascorbic acid was used as positive control.
- Blank: 5ml methanol+100 micro liter DPPH= 0.441

Tab 3.4: Dilution of papaya leaf extract and reference standard

Absorbance of Ascorbic Acid (Standard):	**Absorbance of Papaya extract:**
1. 1ml AA+ 4 ml methanol=0.018	1. 1ml ex+ 4 ml methanol=0.382
2. 2 ml AA+3ml methanol= 0.015	2. 2ml ex+3ml methanol=0.391
3. 3 ml AA+2ml methanol= 0.016	3. 3ml ex+2ml methanol=0.375
4. 4ml AA+1ml methanol= 0.017	4. 4ml ex+1ml methanol=0.310
5.5ml AA+0ml methanol= 0.015	5. 5ml ex+0ml methanol=0.263

Chapter- 4
Result

4.1 Result and Discussion of Antimicrobial Activity

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.

The methanolic extract of *Carica papaya* has observed moderate activity against bacteria. The zone of inhibition has been compared to standard kanamycine. Each disc of standard contains 30 µg/ml kanamycine. There were 3 different concentration of methanolic extract of sample and these were 50µg/ml, 100µg/ml, 150µg/ml. Different zone of inhibition was observed for these 3 disc. The zone of inhibition of *Carica papaya* disc and reference standard is given below:

Tab 4.1: Zone of inhibiton of *Carica papaya* disc and reference standard

Test organism	0.025g/ml	0.05g/ml	0.075g/ml	Kanamycine
Escherichio coli	9.5mm	10mm	10mm	37
Shigella dysenteria	9mm	8.5mm	8mm	37
Salmonella parathyphi	-	-	3.4mm	34
Staphylococcus aureus	8.5mm	9mm	9.5mm	34

4.2 Result and Discussion of DPPH Free Radical Scavenging Activity

The methanolic extract of *Carica papaya* (leaves) were subjected to free radical scavenging activity developed by the method of Brand-Williams *et al.,* 1995. Here ascorbic acid was used as reference standard.

Blank: 5ml methanol+100 micro liter DPPH, absorbance 0.441.

Tab 4.2: Absorbance of standard

Concentration of Ascorbic Acid (mg/ml)	Absorbance
0.02	0.018
0.04	0.015
0.06	0.016
0.08	0.017
0.1	0.015

Tab 4.3: % Inhibition of standard:

Concentration(mg/ml)	% Inhibition
0.02	95.91
0.04	96.6
0.06	96.37
0.08	96.37
0.1	96.6

Tab 4.4: Absorbance of *Carica papaya* leaves extract

Concentration of Ascorbic Acid (mg/ml)	Absorbance
0.02	0.382
0.04	0.391
0.06	0.375
0.08	0.310
0.1	0.263

Tab 4.5: % Inhibition of *Carica papaya* leaves extract

Concentration(mg/ml)	% Inhibition
0.02	13.37
0.04	11.33
0.06	14.96
0.08	29.70
0.1	40.36

The antioxidant activity of *Carica papaya* was found. The plant extract has free radical scavenging activity which has been compared with standard Ascorbic acid. The graphical comparison of free radical scavenging activity of *Carica papaya* and Ascorbic acid given below:

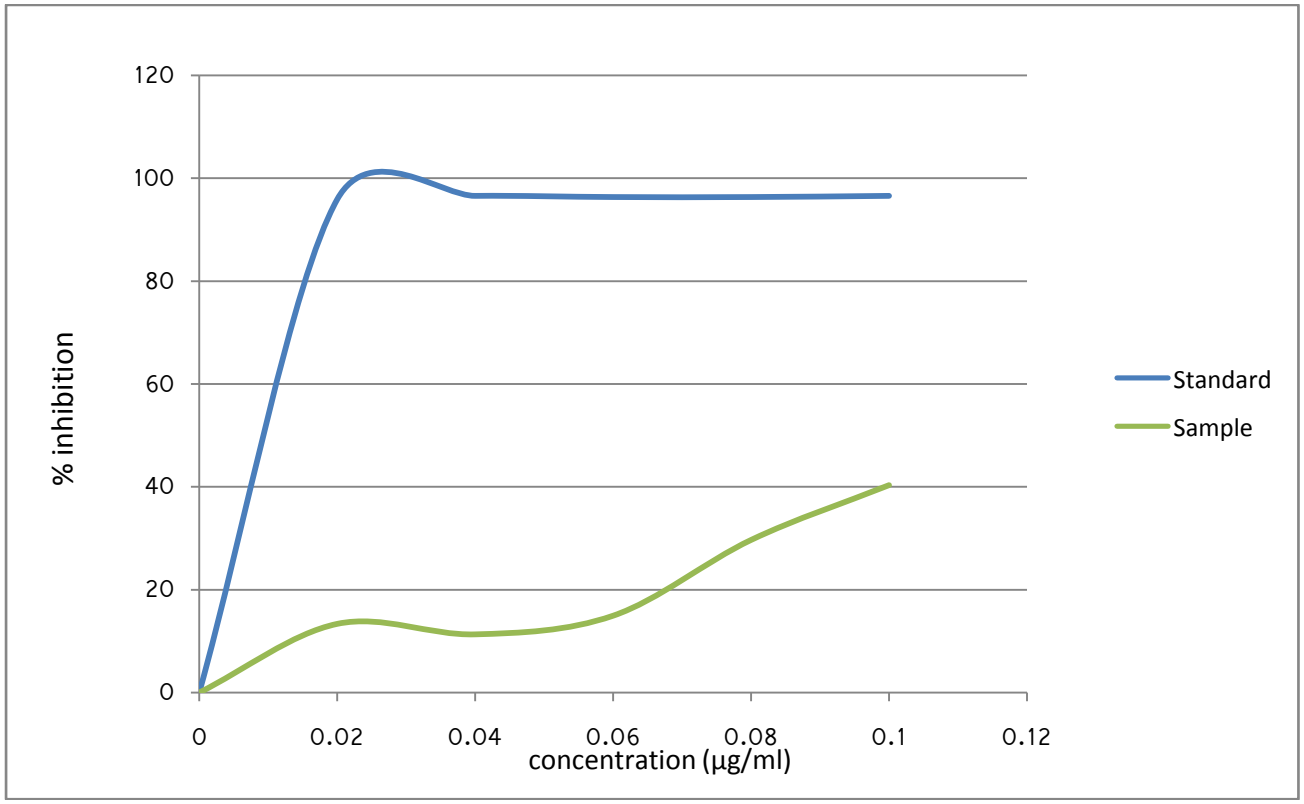


Figure 4.1: Free Radical Scavenging Activity of *Carica papaya* extract and Asorbic acid

Methanolic extract of *Carica papaya* contain such compound which may has powerful or moderate antioxidant activity. The graph shows that ascorbic acid that used as standard has very powerful antioxidant activity. The test sample methanolic extract of *Carcica papaya* also shows moderate antioxidant activity

Chapter-6
Conclusion

6.1 Conclusion

The study was about the antimicrobial activity and the antioxidant activity of *Carica papaya* leaves. Only the methanolci extracts were used to perform this tests. The result of this study was positive and found activity both antibacterial and antioxidant. Isolation of pure compounds from the extract is not done but hope to do it in future.

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